ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma.201701170

Sequentially Responsive Shell-Stacked Nanoparticles for Deep Penetration into Solid Tumors

Jinjin Chen, Jianxun Ding, Yucai Wang, Jianjun Cheng,* Shengxiang Ji, Xiuli Zhuang, and Xuesi Chen**

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Experimental Section

Materials: The amino-terminal methoxy poly(ethylene glycol) (mPEG-NH_2, number-average molecular weight (M_n) = 5000.0 g mol^{-1}) was prepared as our previous work.[1] *N*(ε)-benzyloxycarbonyl-L-lysine (ZLL), L-phenylalanine (LP), and L-cystine (LC) were obtained from GL Biochem, Ltd. (Shanghai, P. R. China). ZLL NCA, LP NCA, and LC NCA were synthesized according to the previously reported protocol.[2] Dimethylmaleic anhydride (DMMA) was purchased from TCI (Tokyo, Japan). *n*-Hexylamine was purchased from Sigma-Aldrich (Saint Louis, MO, USA). *N,N*-Dimethylformamide (DMF) was pretreated with calcium hydride (CaH_2) and then purified by distillation at 45 °C under reduced pressure. Doxorubicin hydrochloride (DOX·HCl) was obtained from Beijing Huafeng United Technology Co., Ltd. (Beijing, P. R. China). Fluorescein isothiocyanate (FITC), hydrobromic acid/acetic acid solution (HBr/HAc, 33.0 wt.%), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from

Sigma-Aldrich (Missouri, USA). Cyanine5.5 (Cy5.5)-NHS ester was obtained from Lumiprobe Corporation (Broward, FL, USA). Cell culture products, including Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), were bought from Gibco (Grand Island, NY, USA). LysoTracker Green was purchased from Molecular Probes (Eugene, OR, USA). Anti-survivin and anti-caspase-3 antibodies were purchased from Abcam (Cambridge, UK). The deionized water was prepared through a Milli-Q water purification equipment (Millipore Co., MA, USA).

Characterizations: Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on an AV-300 NMR spectrometer (Bruker, Karlsruhe, Germany) in deuterated trifluoroacetic acid (TFA-*d*) and water (D_2O). Fourier-transform infrared (FT-IR) spectra were recorded on a Bio-Rad Win-IR instrument (Bio-Rad Laboratories Inc., Cambridge, MA, USA) using potassium bromide (KBr) method. Dynamic light scattering (DLS) measurement was performed on a WyattQELS instrument with a vertically polarized He−Ne laser (DAWN EOS, Wyatt Technology Co., Santa Barbara, CA, USA). The scattering angle was fixed at 90°. Transmission electron microscope (TEM) measurement was performed on a JEOL JEM-1011 TEM (Tokyo, Japan) with an accelerating voltage of 100 kV. The zeta potential of nanoassembly was detected by Zeta PALS (Brookhaven instruments corporation, New York, USA). The CD experiments were conducted on a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, UK).

Preparation of Methoxy Poly(ethylene glycol)-block-*Poly(L-lysine) (mPEG*-b-*PLL):* Methoxy poly(ethylene

glycol)-*block*-poly(*N*(ε)-benzyloxycarbonyl-L-lysine) (mPEG-*b*-PZLL) was synthesized by the ring-opening polymerization (ROP) of ZLL NCA using mPEG-NH_2 as a macroinitiator. Typically, dried mPEG-NH_2 (1.0 g, 0.2 mmol) and ZLL NCA (1.5 g, 5.0 mmol) were dissolved in 15.0 mL of dried DMF in a flame-dry flask. The polymerization was performed at 25 °C for three days. Then, the solution was precipitated into 100.0 mL of diethyl ether. The obtained solid was dried under vacuum at room temperature (Yield: 91.3%). The degree of polymerization (DP) of PZLL was determined to be 25 by ^{1}H NMR spectra (Figure S1, SI). The polydispersity index (PDI) and M_n of the block polymer were determined to be 1.28 and 30100 g mol^{-1} by gel permeation chromatography (GPC), respectively.

$mPEG_{113}$-*b*-PLL_{25} was prepared by removing the benzyloxycarbonyl group of $mPEG_{113}$-*b*-$PZLL_{25}$. Briefly, 1.0 g of $mPEG_{113}$-*b*-$PZLL_{25}$ was dissolved in 10.0 mL of TFA. Then, 3.0 mL of 33.3 wt.% HBr/HAc solution was added. After stirred for 1 h at room temperature, the solution was precipitated into 100.0 mL of diethyl ether. The obtained product was dissolved in water, dialyzed (molecular weight cut-off (MWCO) = 3500 Da) against water for three days, and then lyophilized with a yield of 74.7%.

Preparation of Dimethylmaleic Anhydride-Modified Methoxy Poly(ethylene glycol)-block-*Poly(L-lysine) (Shell-DMMA):* The shell was prepared by the reaction between $mPEG_{113}$-*b*-PLL_{25} and dimethylmaleic anhydride (DMMA). In detail, the aqueous solution of $mPEG_{113}$-*b*-PLL_{25} was first adjusted to pH 8.5 using 1.0 M sodium hydroxide (NaOH) aqueous solution. Two times amount of DMMA was added to the

solution gradually, and the pH was also kept in the range of 8 − 9 using 1.0 M NaOH aqueous solution. When pH was constant, the reaction was continued at room temperature for another 12 h. Then, the solution was dialyzed (MWCO = 3500 Da) against NaOH aqueous solution (pH 8 − 9) for another 12 h and lyophilized. The succinic anhydride (SA)-modified shell (Shell-SA) was prepared in the same route. The substitution degree (DS) of DMMA was calculated to be about 80% by the area of peak g and g' in ^{1}H NMR spectrum in Figure S3.

Synthesis of Poly(L-lysine)−Poly(L-phenylalanine-co-L-cystine) (PLL−P(LP-co-LC)): PLL−P(LP-*co*-LC) was synthesized through the ROP of ZLL NCA, and sequential LP NCA and LC NCA with *n*-hexylamine as a initiator, and subsequent deprotection of benzyloxycarbonyl group. Typically, ZLL NCA (1.5 g, 6.7 mmol) was dissolved by 50.0 mL DMF in a dried and nitrogen (N_2)-filled flask. Then *n*-hexylamine (90 μL, 0.67 mmol) was added to the above solution and stirred for polymerization. After three days of reaction, LP NCA (1.3 g, 6.7 mmol) and LC NCA (1.0 g, 3.3 mmol) were put into the mixture and stirred for another three days. At the end of the reaction, the mixture was poured into 500.0 mL of dried ethyl ether, and the precipitation was collected. The product was dissolved in 10.0 mL of trifluoroacetic acid (TFA), and then 3.0 mL of HBr/HAc solution was added for 1 h of reaction. The above solution was then poured into 100.0 mL of dried ethyl ether and then filtered to obtain the coarse product. At last, the final product of (PLL−P(LP-*co*-LC) was purified by dialysis (MWCO = 3500 Da) and collected by freeze drying with the yield of 71.3%. Figure S4 showed ^{1}H NMR spectra and

ascription of peaks in the spectra of the polymers. FT IR spectra (Figure S5) also proved the generation of polypeptide block from the appearances of the typical amide bonds at 1654 cm^{-1} ($\nu_{C=O}$) and 1544 cm^{-1} ($\nu_{C(O)-NH}$).

Exploration of Suitable Weight Ratio for Formation of Thick Shell in SNP: 1.0 mg of core was dissolved in 10.0 mL of phosphate-buffered saline (PBS) at pH 7.4. Then, the DMMA-modified shell (Shell-DMMA) or SA-modified shell (Shell-SA) was added to the solution in the weight ratio of 1, 2, 3, and 4 to the core, respectively. Then the sizes of the formed nanoparticles were measured by DLS.

Preparation of mPEG-b-PLL/DMMA@PLL−P(LP-co-LC) as SNP: SNP was prepared by facilely mixing mPEG-*b*-PLL/DMMA solution and PLL−P(LP-*co*-LC) solution in a weight ratio of 2:1. The zeta potential of nanoassembly was detected to be −7.4 ± 1.1 mV by Zeta PALS. The morphology of nanoassembly was characterized by DLS and TEM.

Analyses of Polypeptide Conformations by Circular Dichroism (CD): The CD experiments were conducted on a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, UK). The samples of mPEG-*b*-PLL, Shell-DMMA, Shell-SA, and PLL−P(LP-*co*-LC) were prepared at concentrations of 0.01 mg ml^{-1} at pH 7.4. The solution was placed in the sample cell with a path length of 1.0 cm. The mean residue molar ellipticity was calculated by Equation 1:

$$\text{Ellipticity } (\text{cm}^2\ \text{deg dmol}^{-1}) = \frac{\text{millidegrees} \times \text{mean residue weight}}{\text{path length in millimetres} \times \text{concentrantion}} \quad (1)$$

The α-helix contents of the samples were calculated by Equation 2 as described in the previous work:[3]

$$\text{Percentage of α-helix (\%)} = \frac{(-[\theta_{222}] + 3{,}000}{39{,}000} \times 100 \quad (2)$$

Preparation of mPEG−P(LP-co-LC) Nanogel (NP): 1.0 g of mPEG-NH_2 was dried *via* azeotrope with toluene, and then dissolved in 100.0 mL of dried DMF. 0.38 g of LP NCA and 0.29 g of LC NCA were put into the mixture and then stirred for three days. The solution was poured into 500.0 mL of ethyl ether, and the final product of methoxy poly(ethylene glycol)−poly(L-cystine-*co*-L-phenylalanine-*co*-L-cystine) (mPEG−P(LP-*co*-LC)) was collected by lyophilization. The yield was calculated to be 75.4%.

Preparations of Fluorescein Isothiocyanate (FITC)-Labeled PLL−P(LP-co-LC) and mPEG−P(LP-co-LC): 100.0 mg of PLL-*b*-P(LP-*co*-LC) and mPEG-*b*-P(LC-*co*-LP) were dissolved in 10.0 mL of DMF. Then 5.0 mg of FITC was added into the solution and reacted with the amino groups in the nanogels. Finally, the solutions were dialyzed (MWCO = 3500 Da) and stored at 4 °C.

Stimuli-Induced Transitions of Morphologies and Charges of NP and SNP: 1.0 mg of NP and SNP were dissolved in 10.0 mL of PBS at pH 7.4 or 6.8. At designated time intervals, the sizes and zeta potentials were measured. Moreover, a drop of the solution was dripped onto a carbon film for TEM imaging after 2 h of incubation.

1.0 mg of NP and SNP were dissolved in 10.0 mL PBS containing 10.0 mM GSH. After 2 h, the sizes were analyzed by both DLS and TEM.

Fabrications and Drug Release Behaviors of NP/DOX and SNP/DOX: 50.0 mg of PLL-*b*-P(LC-*co*-LP) and 50.0 mg of DOX·HCl were dissolved in 20.0 mL of dimethyl sulfoxide (DMSO) and stirred overnight. Then 20.0 mL of PBS was added

to the solution and stirred for another 12 h. The residual free DOX and DMSO were removed by dialysis (MWCO = 3500 Da) against Milli-Q water for 8 h. Finally, PLL−P(LP-*co*-LC)/DOX was used to fabricate SNP/DOX by mixing with mPEG-*b*-PLL/DMMA at a weight ratio of 1.7:1. The DOX loading content of SNP/DOX was determined to be 5.8 wt.% by standard curve method using a fluorescence detector with excitation wavelength (λ_{ex}) of 480 nm and emission wavelength (λ_{em}) of 590 nm. NP/DOX was prepared through the similar way with a DOX loading content of 5.9 wt.%.

NP/DOX or SNP/DOX solution was diluted to 0.1 mg mL^{-1} and transferred into a dialysis bag (MWCO = 3500 Da). The filled bag was immersed in 100.0 mL of PBS at pH 7.4 or 6.8 without or with 10.0 mM GSH. Then the apparatuses were shaken at 37 °C. At predetermined time, 2.0 mL of the external buffer was collected and replaced by the fresh one. The concentration of DOX was determined by a fluorescence detector at 480 nm using a standard curve method.

In Vitro *Tumor Penetration and Cell Uptake to 3D Spheroid Model (3DSM):* A549 cells were seeded in low-attachment 96-well plates at a density of about 1.0 × 10^4 cells per well in 250.0 μL of DMEM and incubated at 37 °C for five days. Then, NP^{FITC} and SNP^{FITC} with the FITC concentration of 5.0 μg mL^{-1} were added into the media and incubated for another 2 or 24 h. The 3DSMs were washed by PBS thrice and put onto a slide glass for confocal laser scanning microscopy (CLSM) observation (ZEISS LSM 780, Germany).

The cell uptakes of NP^{FITC} and SNP^{FITC} to 3DSMs were carried out in the similar way and measured by flow cytometry (FCM). A549 cells were seeded in low-attachment 96-well plates at a density of about 1.0×10^4 cells per well in 250.0 μL of DMEM at 37 °C for five days. Then the medium was replaced by 250.0 μL of DMEM with the pH values of 7.4 and 6.8, respectively. Then NP^{FITC} and SNP^{FITC} at the FITC concentration of 5.0 μg mL^{-1} were added into the media separately and incubated for another 2 or 24 h. Finally, the cells were washed thrice using PBS and suspended in 200.0 μL of PBS. Data for 10,000 gated events were collected, and analyses were performed using a flow cytometer (Millipore, Billerica, USA).

In Vitro *Cell Uptakes and Distributions of NP/DOX and SNP/DOX:* The cells were treated with free DOX, NP/DOX, and SNP/DOX at a final DOX concentration of 10.0 mg L^{-1} in 2.0 mL of complete DMEM at pH 7.4 or 6.8. Additionally, in the group pretreated with GSH, the cells were preincubated with 10.0 mM of GSH for 2 h before the addition of DOX formulations. For FCM detection, after being incubated for another 2 h, the cells in each well were suspended in 1.0 mL of PBS and centrifuged for 4 min at 3000 rpm. After removing the supernatants, the cells were resuspended in 0.3 mL of PBS. Data for 10,000 gated events were collected. For CLSM detection, after incubated for another 2 or 6 h as predetermined, the cells was incubated with LysoTracker Green for 1 h at 37 °C. Afterwards, the cells were washed thrice with PBS and fixed with 4% (*W*/*V*) PBS-buffered formaldehyde. Later, the fixed cells were incubated with DAPI for 5 min at room temperature and then washed with PBS thrice. Finally, the images of cells were observed by a CLSM.

In Vitro *Cytotoxicity Assay:* The cytotoxicities of blank SNP, NP/DOX, and SNP/DOX were analyzed using a MTT assay toward A549 cells *in vitro*. In the blank SNP group, the cells were treated with SNP (0 − 2.5 mg mL^{-1}) in 200.0 μL of complete DMEM. In the NP/DOX and SNP/DOX groups, the cells were treated with NP/DOX or SNP/DOX (0 − 10.0 mg L^{-1} DOX) in 200.0 μL of complete DMEM at pH 7.4 or 6.8. To evaluate the influence of intracellular reduction, the cells were preincubated with GSH (10.0 mM) for 2 h before the addition of DOX-loaded nanocarriers. Then, the cells were subjected to the MTT assay after being incubated for 48 h. The absorbances of the above media were measured at 490 nm on a Bio-Rad 680 microplate reader. The cell viability was calculated based on Equation 3:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (3)$$

In Equation 3, A_{sample} and A_{control} represented the absorbances of sample and control wells, respectively.

As shown in Figure S8, the blank SNP showed no cytotoxicity even with the concentration up to 2.5 mg mL^{-1}. The half-maximal inhibitory concentration (IC_{50}) of SNP/DOX listed in Table S1 decreased from 1.19 ± 0.07 to 0.71 ± 0.12, owing to the enhanced cell uptake after the detachment of shell. Moreover, the GSH-mediated increase of cytotoxicity of both NP/DOX and SNP/DOX further demonstrated the GSH-accelerated DOX release.

Animals and Tumor-Xenografted Model: BALB/c nude mice (male, 18.0 − 20.0 g, 5 − 6 weeks) and Sprague-Dawley (SD) rats (male, 200.0 − 220.0 g, 5 − 6 weeks) were purchased from the Beijing HFK Bioscience Co., Ltd. (Beijing, P. R. China),

and all mice in this work were handled under the protocol approved by the Institutional Animal Care and Use Committee of Jilin University. All efforts were made to minimize suffering. The A549 tumor-bearing nude mouse model was generated by injecting 2.0×10^7 cells in 100.0 μL of PBS into the right flank of the BALB/c nude mice.

In Vivo *Blood Vascular Extravasation, Tumor Penetration, and Cell Uptake of* NP^{FITC} *and* SNP^{FITC}*:* The vascular extravasation of NP^{FITC} and SNP^{FITC} were observed by intravital CLSM as described in the previous work.[4] In detail, mice bearing A549 tumor were intravenously injected with NP^{FITC} and SNP^{FITC} at the FITC dosage of 50.0 μg per kg body weight (μg (kg BW)$^{-1}$). The mice were then anaesthetized with pentobarbital by intraperitoneal injection. An arc-shaped incision was made around the subcutaneous tumor, and the skin flap was elevated without injuring the feeding vessels. The mouse was then directly placed on cover slip. The cover slip was attached with just enough pressure to flatten the tumor surface. All *in vivo* image acquisitions were performed using a Zeiss LSM780 CLSM by a 20× objective. At the end of experiment, the mice were sacrificed. The tumors were collected and cut into 10.0 μm slices. Then the sections were also observed by CLSM using tile scan for evaluation of the tumor distribution. Finally, the collected tumor sections were further stained by DAPI and also observed by CLSM for evaluation of *in vivo* cell uptake to the deep tumor cells.

Pharmacokinetic Studies: SD rats were administrated with free DOX, NP/DOX, or SNP/DOX by tail vein injection. At predetermined time intervals (*i.e.*, 0.3, 0.5, 1,

2, 4, 8, 12, and 24 h), 500.0 μL of blood was collected from the orbital venous plexus, and then heparinized and centrifuged to obtain the plasma. Subsequently, 1.0 mL of acetonitrile was added into the plasma for protein settlement. After centrifugation at 12000 rpm for 5 min, the supernatant was collected and dried under the nitrogen stream. Finally, the obtained residues were redissolved in 300.0 μL of acetonitrile for high-performance liquid chromatographic (HPLC) detection, which were performed on a Waters e2695 HPLC system equipped with Waters 2487 two-channel fluorescence detector and a Symmetry C18 column (Waters, Milford, MA, USA). Acetonitrile−water (30:70, *V*/*V*, pH 3.5) was used as an elution with a flow rate of 1.0 mL min^{-1}. Fluorescence detector was set at 480 nm for excitation and 590 nm for emission, and linked to Breeze software for data analysis.

Biodistribution Assays: The tissue distributions of DOX after intravenous injections of various formulations were qualitatively or semiquantitatively assessed by *ex vivo* DOX fluorescence imaging of major internal organs. Typically, DOX, NP/DOX, and SNP/DOX were administered intravenously to the nude mice bearing A549-xenografted tumors at the DOX dosage of 5.0 mg per kg body weight (mg (kg $BW)^{-1}$). The mice were sacrificed after 12 h. Then the major organs, including the heart, liver, spleen, lung, and kidney, as well as the tumor, were excised and washed with cold normal saline. The *ex vivo* DOX fluorescence imaging was obtained using the Maestro *in vivo* fluorescent imaging system (Cambridge Research & Instrumentation Inc., Woburn, USA). In addition, the average signals were also

semiquantitatively analyzed using ImageJ software (National Institutes of Health, Bethesda, USA).

Evaluations of Maximum Tolerated Doses: The maximum tolerated doses (MTDs) of free DOX, NP/DOX, SNP/DOX, and blank SNP were evaluated in male Kunming mice weighting 30.0 − 35.0 g. All groups (n = 5 per group) received a single-dose by intravenous injection. In the DOX, NP/DOX, and SNP/DOX groups, the final dose of DOX were 5.0, 10.0, or 20.0 mg (kg BW)$^{-1}$. In the blank SNP group, the mice received 125.0, 250.0, or 500.0 mg (kg BW)$^{-1}$ of blank SNP. After a single tail vein injection, the movement, skin, hair, body weights, and survival rates were monitored daily over 10 days in all groups. MTD was defined as the allowance of an average body weight loss of 15% initial weight and causing neither death due to toxic effects nor remarkable changes in the general signs within 10 days after administration.

Biochemical Parameter Analyses: At the end of the MTD study, the blood was collected. The serum was obtained through centrifugation at 3,000 rpm for 5 min. The enzyme-linked immunosorbent assay (ELISA) kits of creatine kinase (CK), alanine aminotransferase (ALT), aspartate transaminase (AST), and blood urea nitrogen (BUN) were purchased from Shanghai Lengton Bopscience Co., Ltd. (Shanghai, P. R. China). All the detections were carried out according to the instructions of above kits.

In Vivo *Therapeutic Evaluation:* When the xenografted A549 tumors grew to about 60 mm^3, the mice were randomly divided into four groups (n = 6 per group) and treated with PBS, free DOX, NP/DOX, or SNP/DOX at the equivalent DOX dose of 5.0 mg (kg BW)$^{-1}$ by intravenous injection on day 0, 4, 8, 12, and 16. Tumor growth

was monitored by measuring the perpendicular diameter of the tumor using calipers. Tumor volume (mm^3) was estimated by the following Equation 4.

$$V = \frac{a \times b^2}{2} \tag{4}$$

In Equation 4, *a* and *b* were the major and minor axes of the tumor measured by a caliper.

Histological Analyses: At the end of all treatments, the tumors were collected and fixed in 4% (*W/V*) PBS-buffered paraformaldehyde overnight and then embedded in paraffin. The paraffin-embedded tissues were then cut into about 5.0 μm slices for hematoxylin and eosin (H&E) staining and immunohistochemical analyses. The H&E staining were detected by a microscope (Nikon Eclipse *Ti*, Optical Apparatus Co., Ardmore, USA).

To analyze the expression of caspase-3 and survivin, the deparaffinized slides were boiled by a microwave oven in 0.01 M sodium citrate buffer at pH 6.0 for antigen retrieval. Subsequently, slides were allowed to cool down for another 5 min in the same buffer. After several washing in PBS and pretreatment with blocking medium for 5 min, slides were incubated with caspase-3 and survivin antibody (Abcam, Cambridge, UK) at 4 °C overnight. After another three times of washing with PBS, the FITC-labeled secondary antibody was used to treat the sections. After the last three times of washing, the samples were observed by CLSM.

Statistical Analyses: All experiments were performed at least three times, and the data were presented as mean ± standard deviation. Differences between experimental groups were assessed by a one-way analysis of variance with statistical software SPSS

17.0 (SPSS Inc., Chicago, USA). *$P < 0.05$ was considered statistically significant, and **$P < 0.01$ and ***$P < 0.001$ were considered highly significant.

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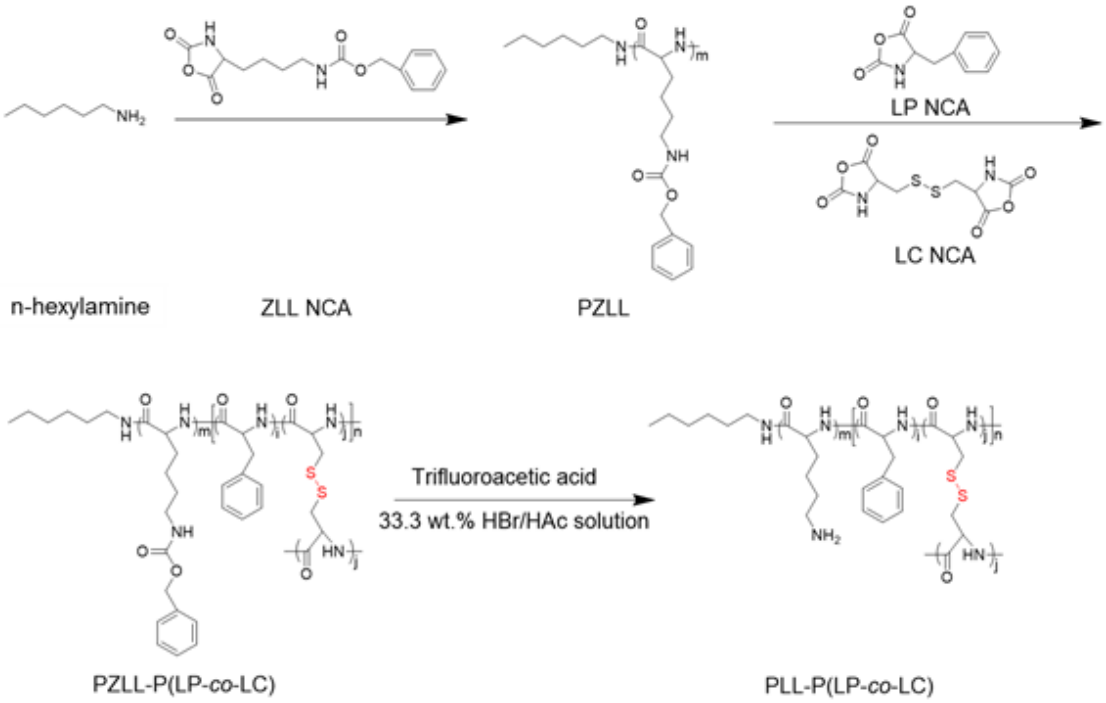

Figure S1. Synthesis pathway for PLL−P(LP-*co*-LC) nanogel.

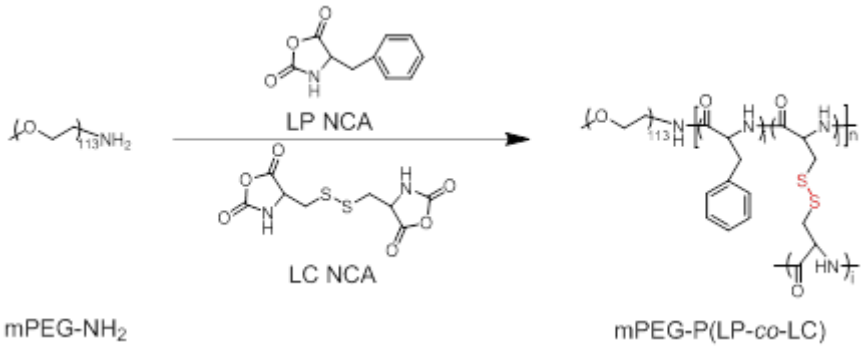

Figure S2. Synthesis proposal of mPEG−P(LP-*co*-LC), *i.e.*, NP.

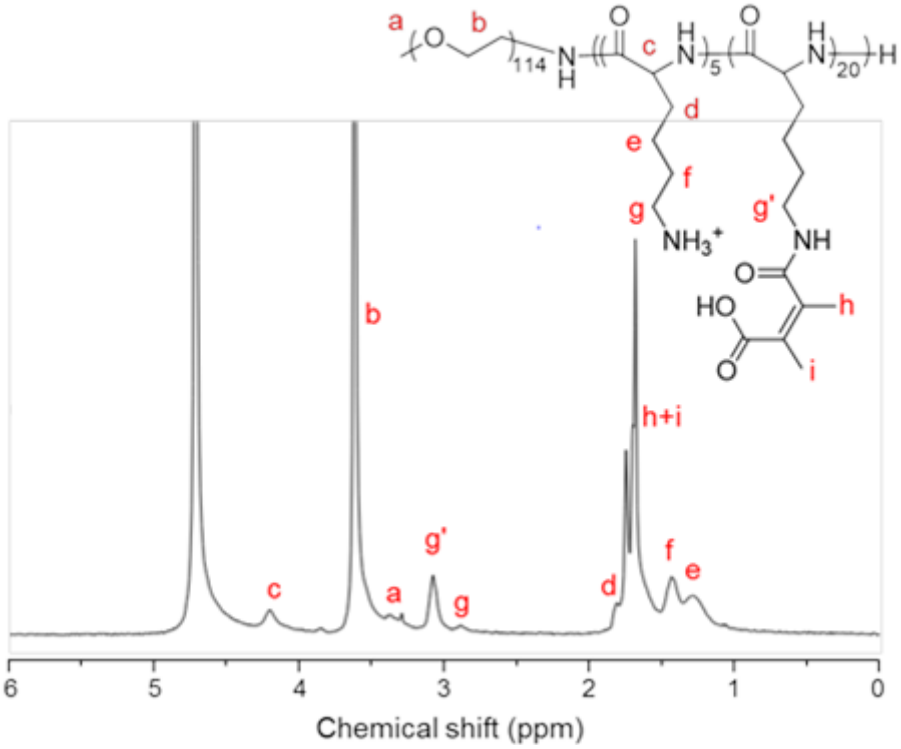

Figure S3. ^{1}H NMR spectrum of Shell-DMMA using D_2O as a solvent.

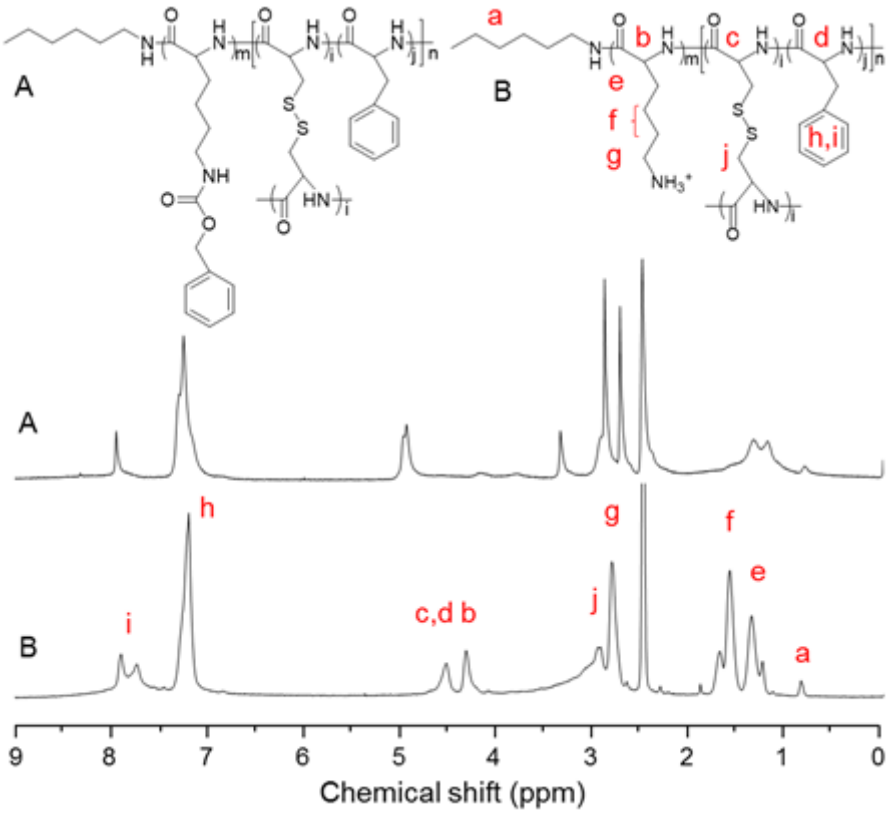

Figure S4. ^{1}H NMR spectra of PZLL−P(LP-*co*-LC) (A) and PLL−P(LP-*co*-LC) (B) using TFA-*d* as a solvent.

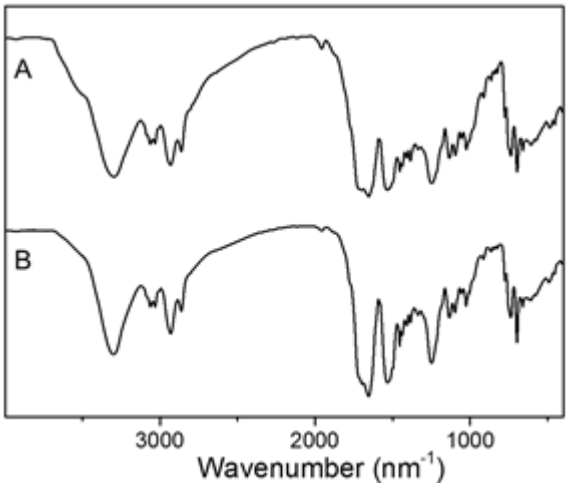

Figure S5. FT-IR spectra of PZLL−P(LP-*co*-LC) (A) and PLL−P(LP-*co*-LC) (B).

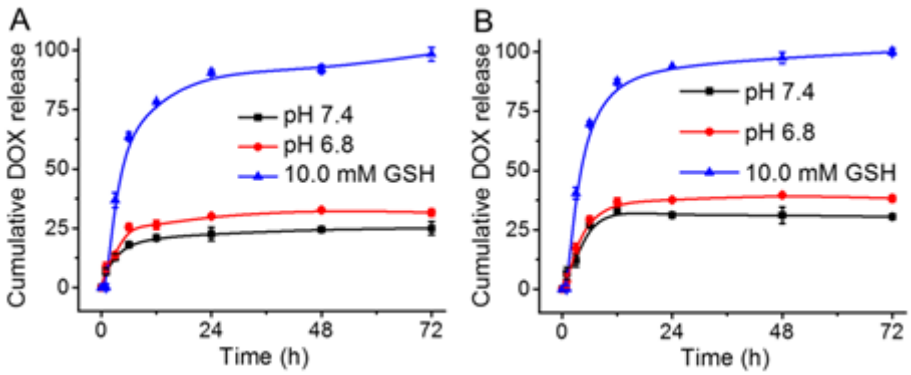

Figure S6. DOX release behaviors of NP (A) and SNP (B) in PBS at pH 7.4 or 6.8 without or with 10.0 mM GSH.

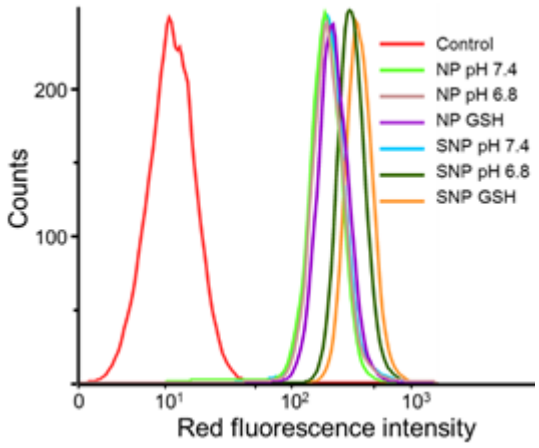

Figure S7. Cell uptake of DOX toward A549 cells after incubated with NP/DOX and SNP/DOX at pH 7.4 or 6.8 without or with 10.0 mM GSH. These results revealed that the pH-promoted DOX uptake and GSH-accelerated DOX release in the SNP/DOX group.

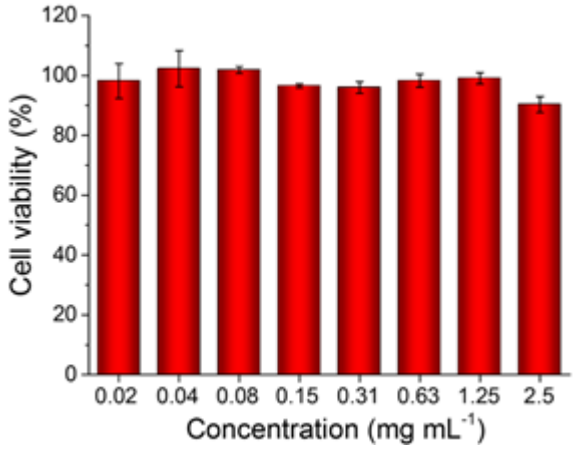

Figure S8. Cytotoxicity of blank SNP at different concentrations.

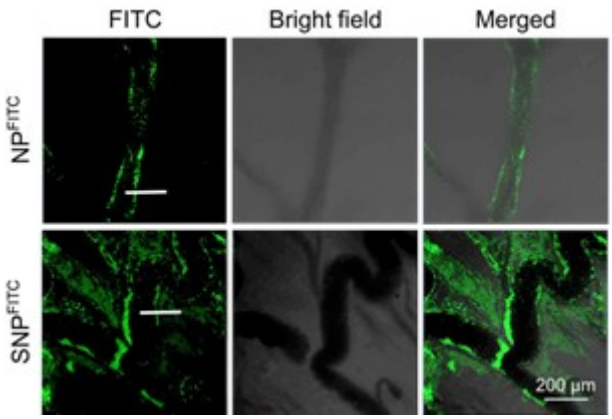

Figure S9. Distribution of NP^{FITC} and SNP^{FITC} along with the blood vessels. The blood vessels were shown to be the shadows in the bright field images.

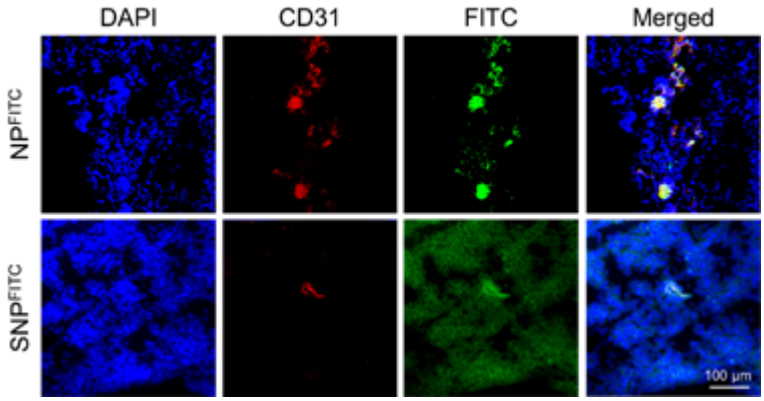

Figure S10. CLSM images of immunofluorescence showing the microdistribution of NP^{FITC} and SNP^{FITC} in tumor tissue at 2 h postinjection.

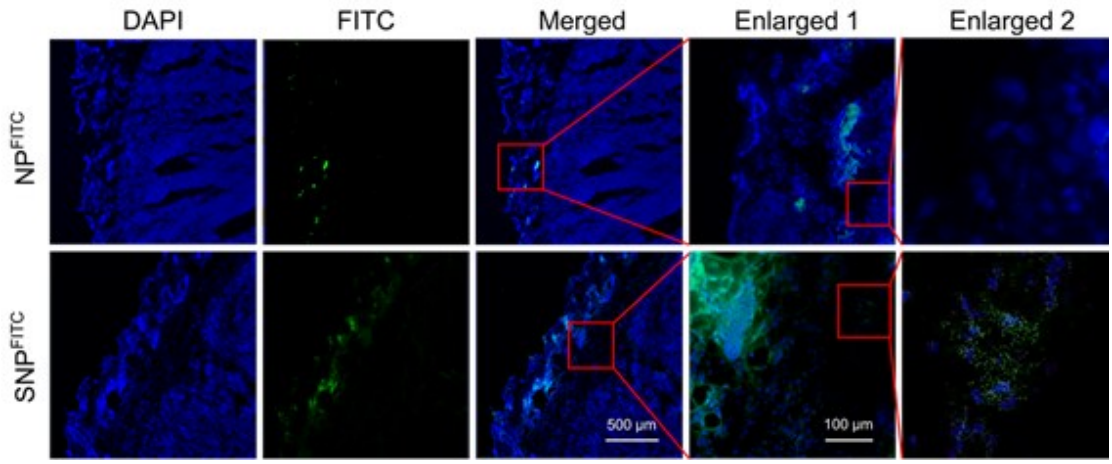

Figure S11. *In vivo* cell uptakes of NP^{FITC} and SNP^{FITC} after tumor penetration. The cell nucleus was stained with DAPI.

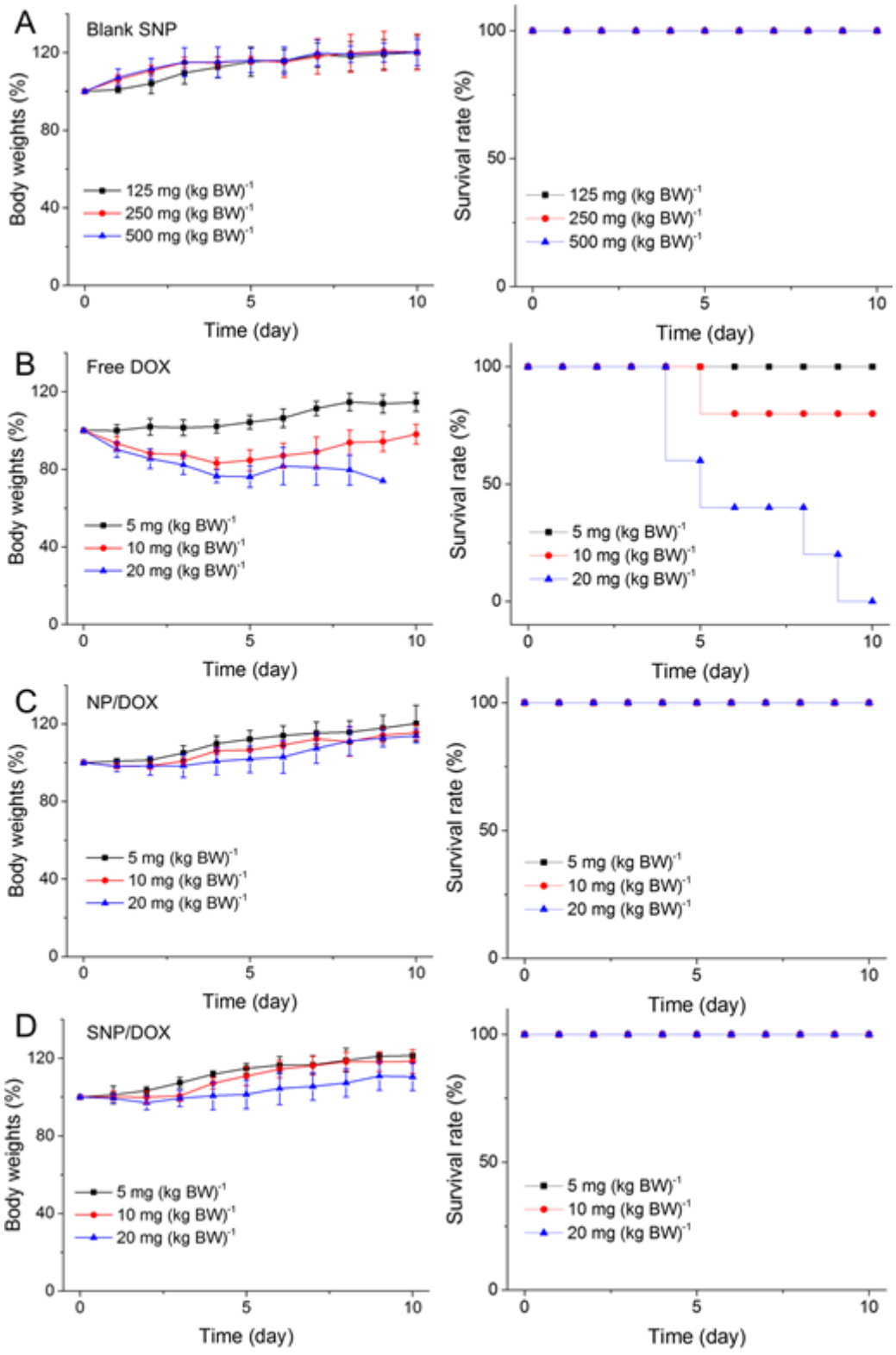

Figure S12. The changes of body weights and survival rates of Kunming mice after treatment with blank SNP (A), free DOX (B), NP/DOX (C), or SNP/DOX (D) at different dosages.

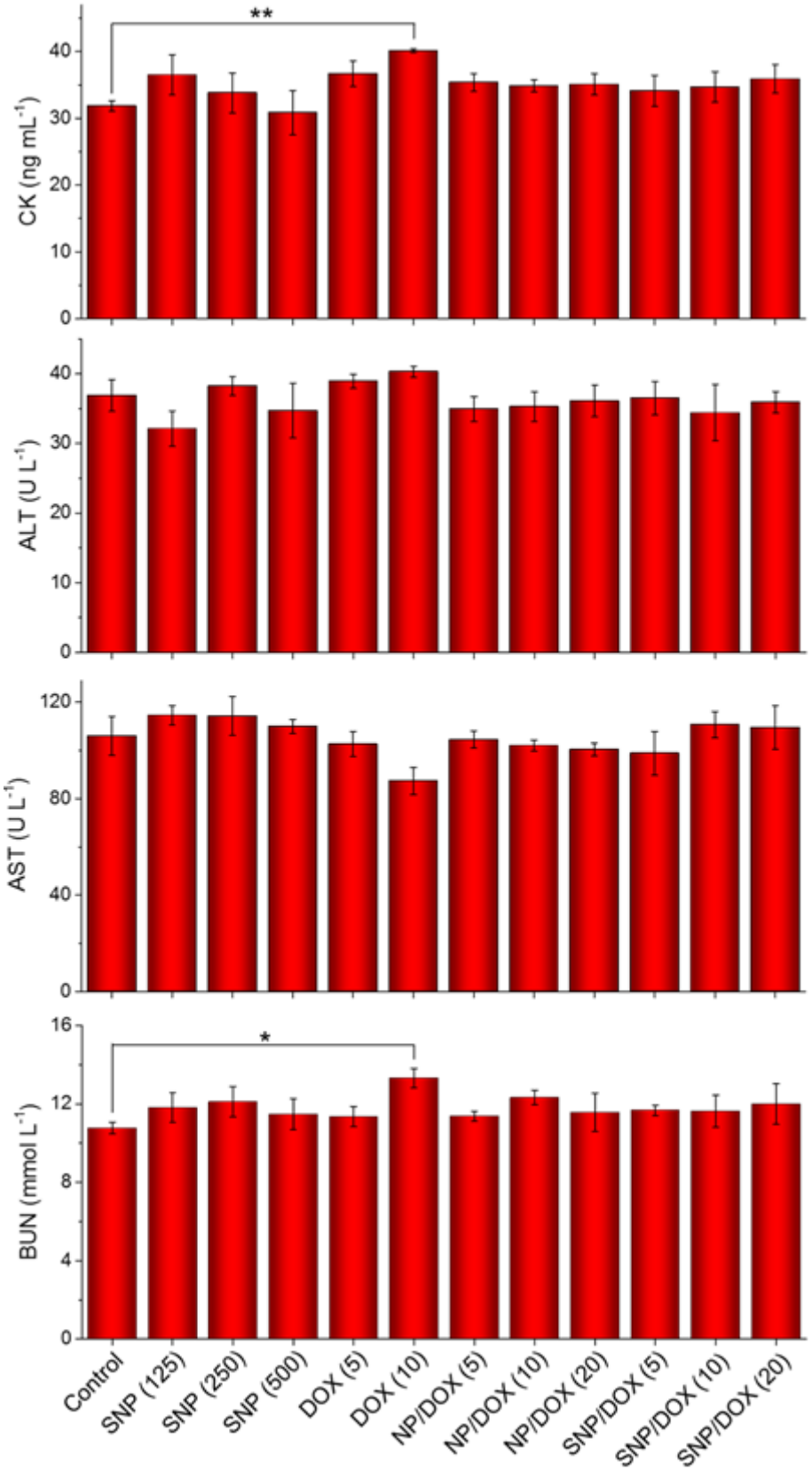

Figure S13. Evaluations of CK, ALT, AST, and BUN by ELISA kits after treatments with different formulations for 10 days. (n = 3; *P < 0.05, **P < 0.01, ***P < 0.001).

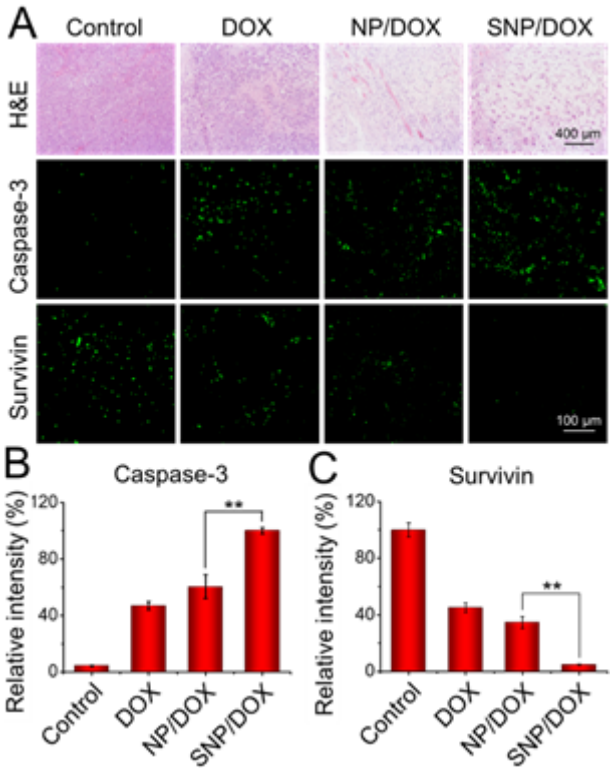

Figure S14. Histopathology and Immunofluorescence Analyses. (A) *Ex vivo* histopathological analyses of tumor sections after treatment with PBS, DOX, NP/DOX, or SNP/DOX. (B and C) Relative caspase-3 (B) and survivin intensities (C) of tumor sections collected from mice treated with PBS, DOX, NP/DOX, or SNP/DOX. Data were represented as mean ± standard deviation (n = 3; *P < 0.05, **P < 0.01, ***P < 0.001).

Table S1. IC_{50}s of NP/DOX and SNP/DOX to A549 cells after incubation for 24 h at pH 7.4 or 6.8 without or with preincubation of 10.0 mM GSH.

		IC_{50} (mg mL^{-1})	
		Without GSH	Preincubated with GSH
NP/DOX	pH 6.8	1.01 ± 0.09	0.65 ± 0.09
	pH 7.4	1.09 ± 0.03	0.85 ± 0.07
SNP/DOX	pH 6.8	0.71 ± 0.12	0.24 ± 0.02
	pH 7.4	1.19 ± 0.07	0.50 ± 0.10