Supporting Information

In Situ Real-Time Trace of Hierarchical Targeting Nanostructures in Drug Resistant Tumor Using Diffuse Fluorescence Tomography

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Materials and Methods

1. Materials

3-Aminophenylboronic acid (PBA-NH_2) monohydrate was purchased from Nanjing Kangmanlin Chemical Industry Co. Ltd. (Nanjing, China). 2-Aminoethyl methacrylate hydrochloride (AMA), lactobionolactone was purchased from Tianjin Heowns Biochem Technology Co. Ltd. (Tianjin, China). Boron-dipyrromethene dye-conjugated chain transfer agent (BODIPY-RAFT) was prepared by our group according to the previously reported method.[1] Indocyaninegreen (ICG) was purchased from Liaoning Tianyi Biological Pharmaceutical Co., Ltd. (Liaoning, China). 3[4,5-Dimethylthiazol-2-yl]-2,5-diphenylterazolium bromide (MTT) and 4',6-diamidino-2-phenylindole (DAPI) were purchased from J&K China Chemical Ltd. (Beijing, China). All solvents used were of analytical grade without further purification.

2. Synthesis of poly(carboxybetaine acrylate)-*block*-poly(2-(acrylamido) glucopyranose) (pCBAA-*b*-pAGA).

2.1. Synthesis of poly(carboxybetaine acrylate) (pCBAA)

pCBAA was synthesized by RAFT polymerization using CBAA as monomer, BODIPY-RAFT as RAFT agent and 4,4'-azobis(4-cyanovaleric acid) (ACVA) as initiator **(Scheme S1**A**)**. Briefly, CBAA (0.343 g, 1.5 mmol), BODIPY-RAFT (0.234 g, 0.05 mmol) and ACVA (0.003 g, 0.01 mmol) were dissolved in methanol and charged into a polymerization tube. The blending was purged with N_2 for 30 min and the reaction was conducted for 10 h at 55 °C. After quenching the reaction in an ice bath for 5 min, the mixture was poured into a large excess of THF for polymer precipitation. By determining the molar ratio of CBAA monomer

to BODIPY-RAFT, homopolymer pCBAA was obtained and named as $pCBAA_{30}$.

2.2. Synthesis of 2-(acrylamido) glucopyranose) (AGA)

AGA was synthesized by the previously reported method.[2] Briefly, D-glucosamine hydrochloride (10.0 g, 4.64×10^{-2} mol) and potassium carbonate (6.41 g, 4.64×10^{-2} mol) were dissolved in methanol (250 mL) in a 500 mL single neck round-bottom flask. Acryloyl chloride (3.77 g, 4.17×10^{-2} mol) was added dropwise into the mixture under vigorous stirring in ice bath. The mixture was stirred for 30 min and then reacted for another 3 h at 25 °C. The crude mixture was concentrated to be off-white slurry, and purified via silica gel column chromatography using ethyl acetate/methanol (v/v, 4:1) as eluents. The pure product was obtained as a white solid.

2.3. Synthesis of poly(carboxybetaine acrylate)-*block*-poly(2-(acrylamido) glucopyranose) (pCBAA-*b*-pAGA)

Copolymer pCBAA-*b*-pAGA was synthesized by RAFT polymerization **(Scheme S1**A**)**. RAFT polymerization was performed at [AGA]:[RAFT agent]:[AVCA] = 10:1:0.2 using AGA as monomer, homopolymer $pCBAA_{30}$ as macroRAFT agent, ACVA as initiator, and CH_3OH/H_2O (v/v, 3:1) as the mixed solvents. After purging with N_2 for 30 min, the reaction system was conducted for 12 h at 55 °C. After quenching the reaction in an ice bath for 5 min, the resultant copolymer was obtained by precipitating into excess of THF. By determining the molar ratio of AGA monomer to $pCBAA_{30}$ macroRAFT, copolymer pCBAA-*b*-pAGA was obtained and named as $pCBAA_{30}$-*b*-$pAGA_{10}$ (**Table S**1).

3. Synthesis of poly(2-lactobionamidoethyl methacrylate–*block*-poly(3-acrylamidophenylboronic acid)) (pLAMA-*b*-pAAPBA).

3.1. Synthesis of 2-lactobionamidoethyl methacrylate (LAMA)

Firstly, LAMA was synthesized according to the previously reported method.[3] Lactobionolactone (10.0 g, 2.94 × 10^{-2} mol) was dissolved in methanol at 40 °C and then cooled to 25 °C before the addition of AMA (10.0 g, 6.04 × 10^{-2} mol), triethylamine (10.0 mL) and hydroquinone (0.25 g, 1.4 × 10^{-3} mol). The reaction was carried out for 5 h, then concentrated and precipitated into isopropanol. The pure product was obtained after being filtered, washed with isopropanol and dried.

3.2. Synthesis of poly(2-lactobionamidoethyl methacrylate) (pLAMA)

pLAMA was synthesized by RAFT polymerization using LAMA as monomer, BODIPY-RAFT as RAFT agent and ACVA as initiator **(Scheme S**1B**)**. Briefly, LAMA (0.700 g, 1.5 mmol) was dissolved in H_2O and charged into a polymerization tube. BODIPY-RAFT (0.234 g, 0.05 mmol) and ACVA (0.003 g, 0.01 mmol) dissolved in ethanol were added into the tube, then the blending was purged with N_2 for 30 min and the reaction system was conducted for 12 h at 55 °C. After quenching the reaction in an ice bath for 5 min, the mixture was poured into a large excess of methanol for polymer precipitation. By determining the molar ratio of LAMA monomer to BODIPY-RAFT, homopolymer pLAMA was obtained and named as $pLAMA_{30}$.

3.3. Synthesis of 3-acrylamidophenylboronic acid (AAPBA)

AAPBA was prepared using the modified method described by Lee et al.[4] Briefly, PBA-NH_2 monohydrate (5.0 g, 3.22 × 10^{-2} mol) and sodium bicarbonate (5.0 g, 5.95 × 10^{-2} mol) were dissolved in 45 mL of H_2O and tetrahydrofuran (THF) (v/v, 2:1) and cooled to 0 °C. Acryloyl chloride (5 mL, 6.25 × 10^{-2} mol) was added dropwise and the reaction was performed under

vigorous stirring for another 2 h at 25 °C. The obtained solution was extracted with acetic ether and the organic phase was concentrated to a dry crude product. Subsequently, the crude product was re-crystallized twice in hot water (80 °C) to obtain crystals as the pure product.

3.4. Synthesis of poly(2-lactobionamidoethyl methacrylate–*block*-poly(3-acrylamidophenylboronic acid)) (pLAMA-*b*-pAAPBA)

Copolymer pLAMA-*b*-pAAPBA was synthesized by RAFT polymerization **(Scheme S**1B**)**. RAFT polymerization was performed at [AAPBA]:[RAFT agent]:[AVCA] = 10:1:0.2 using AAPBA as monomer, homopolymer $pLAMA_{30}$ as macroRAFT agent, ACVA as initiator, and DMF/water (v/v, 1:1) as the mixed solvent. After purging with N_2 for 30 min, the reaction system was conducted for 12 h at 55 °C. After quenching the reaction in an ice bath for 5 min, the resultant copolymer was obtained by precipitating into excess acetic ether, washing with water, and drying under vacuum. By changing the molar ratio of AAPBA monomer to $pLAMA_{30}$ macroRAFT, three distinct block copolymers pLAMA-*b*-pAAPBA were obtained and named as $pLAMA_{30}$-*b*-$pAAPBA_{10}$, $pLAMA_{30}$-*b*-$pAAPBA_{15}$ and $pLAMA_{30}$-*b*-$pAAPBA_{20}$, respectively (**Table 1**).

4. Preparation of nanoassembly

In a typical procedure, $pCBAA_{30}$-*b*-$pAGA_{10}$ (10 mg, 1.2 μmol) and $pLAMA_{30}$-*b*-$pAAPBA_{20}$ (21 mg, 1.2 μmol) were dissolved in 2 mL of the mixed solvents DMSO/H_2O (v/v, 1:3), and then 8 mL of PBS buffer (pH 7.4) was added dropwise into the mixture solution under vigorous stirring. The resultant nanoparticle solution was transferred into a dialysis tube (MWCO 3500 Da) and dialyzed against PBS buffer (pH 7.4) for 24 h. The organic solvent was removed by replacing PBS buffer (pH 7.4) every 3 h and the resultant nanoparticles were

denoted as pCA/pLB Np3.

5. Shell detachable behavior of pCA/pLB nanoassembly.

pCA/pLB Np3 (10 mg/mL) was suspended in PBS buffer at pH 6.5 or 7.4. At a predetermined time intervals, 1 mL of the solution was taken outafter the centrifugation for UV-vis measurement (λ = 506 nm) and replenished with the same volume of fresh medium. Each sample was analyzed triplicate and results were reported as mean ± standard deviation (n = 3).

6. Loading drug

6.1. Loading Doxorubicin (DOX)

DOX (4 mg), $pCBAA_{30}$-*b*-$pAGA_{10}$ (10 mg) and $pLAMA_{30}$-*b*-$pAAPBA_{20}$ (20 mg) were dissolved in 2 mL DMSO/H_2O (v/v, 1:3). 8 mL of PBS buffer (pH 7.4) was added dropwise into the mixture under vigorous stirring and the resultant mixture solution was stirred for another 24 h in the darkness. DMSO was removed via dialysis (MWCO 3500) against PBS buffer (pH 7.4) for 24 h in the darkness, and fresh buffer was used as replacement every 3 h and the resultant nanoparticles were denoted as DOX-loaded pCA/pLB Np3.

To evaluate drug loading and encapsulation efficiency, 2.0 mL of DOX-loaded pCA/pLB Np3 suspension was centrifuged and the supernatant was determined at 485 nm by a UV-vis spectrometer. The amount of loaded DOX was calculated according to the total volume of dialyzed nanoparticle dispersions. Drug loading capacity (LC) and encapsulation efficiency (EE) were estimated using the following equations:

$$\mathrm{LC\%} = \frac{\text{weight of DOX in NPs}}{\text{weight of NPs}} \times 100\% \qquad (1)$$

$$\mathrm{EE\%} = \frac{\text{weight of DOX in NPs}}{\text{weight of total DOX}} \times 100\% \qquad (2)$$

6.2. Loading ICG

To explore the trace of nanocarriers *in vivo*, ICG was entrapped into the nanoparticles to act as the tracer agent of nanocarriers. ICG (2 mg), $pCBAA_{30}$-*b*-$pAGA_{10}$ (5 mg, 0.06 μmol) and $pLAMA_{30}$-*b*-$pAAPBA_{20}$ (10 mg, 0.06 μmol) were dissolved in 1 mL of DMSO/H_2O (v/v, 1:3). 4 mL of PBS buffer (pH 7.4) was added dropwise into the mixture under vigorous stirring and the resultant mixture solution was stirred for another 24 h in the darkness. DMSO was removed via dialysis (MWCO 3500) against PBS buffer (pH 7.4) for 24 h in the darkness, and fresh buffer was used as replacement every 3 h and the resultant nanoparticles were denoted as ICG-loaded pCA/pLB Np3.

To evaluate drug loading and encapsulation efficiency, 1.0 mL of ICG-loaded pCA/pLB Np3 suspension was centrifuged and the supernatant was determined measured at 784 nm by fluorescence spectrometer. LC and EE were estimated using above mentioned equations (1) and (2).

7. Characterization of copolymers and nanoassembly.

^1H NMR spectra were recorded at 25 °C using a Varian-plus 400 NMR spectrometer. The morphology of nanoparticles was examined using transmission electron microscopy (TEM, FEI, Tecnai G2 F20). Hydrodynamic diameter (D_H) and zeta potential were characterized by dynamic light scattering (DLS) using a Malvern Zetasizer Nano S apparatus equipped with a 4.0 mV laser operating at λ = 636 nm. All measurements were performed at a scatting angle of 90º. UV absorption and fluorescence emission spectra were determined using a UV-vis spectrometer (UV-2550) and fluorescence spectrophotometer (RF-5301PC) from Shimadzu, respectively.

8. *In vitro* DOX release.

5 mL of DOX-loaded pCA/pLB Np3 suspension (1 mg/mL) was transferred to a dialysis tube (MWCO 3500) and dialyzed against 20 mL PBS buffer (pH 5.4, 7.4 and pH 7.4 with 1 mg/mL of glucose, respectively) under shaking (100 rev/min). At specific time intervals, 1 mL of dialyzed medium was withdrawn for UV-vis measurement (λ = 485 nm) and replenished with the same volume of fresh medium. Each sample was analyzed triplicate and results were reported as mean ± standard deviation (n = 3).

9. Treatment of HepG2 and HepG2/R with naked DOX and DOX-loaded pCA/pLB

To identification of HepG2/R resistance to naked DOX treatment, HepG2 and HepG2/R cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in 5% CO_2 at 37 °C. Cells were seeded in a 12-well plate at 1×10^5 cells per well. Then, the cells were incubated with naked DOX for 3 h. The cells were harvested by centrifuging at 1 000 rpm for 5 min at 4 °C and washed with PBS. After three cycles of washing and centrifugation, the cells were resuspended in 1 mL 4% paraformaldehyde. The DOX content in cells was determined using a flow cytometer (Becton Dickinson FACS Calibur).

The uptake of DOX-loaded pCA/pLB by HepG2 and HepG2/R was determined by flow cytometer and confocal laser scanning microscope (CLSM, TCS SP8). Cells were seeded in a 12-well plate at 1×10^5 cells per well. Then, the cells were incubated with DOX-loaded pCA/pLB for 3 h. For flow cytometer, cells were washed, digested and resuspended in 1 mL 4% paraformaldehyde. The DOX-loaded pCA/pLB content in cells was determined using a flow cytometer. For CLSM, cells were washed three times with PBS buffer (pH 7.4), fixed with 4%

paraformaldehyde for 10 min and then sequentially stained with DAPI for 10 min. The DAPI fluorescence (blue), DOX fluorescence (red) and nanoparticle fluorescence (green) were examined by CLSM.

To identify the mechanism of DOX-loaded pCA/pLB Np3 internalization into HepG2/R cells, cells were seeded in 12-well plates at 1×10^5 cells per well. Then, the cells were pretreated with sucrose, nystatin, wortmannin, amiloride and methyl-β-cyclydextrin, respectively, as previously reported.[5] Then, cells were co-incubated with DOX-loaded pCA/pLB Np3 for 3 h. Then, cells were washed, trypsinized, collected and resuspended in 1 mL 4% paraformaldehyde. The DOX-loaded pCA/pLB content in HepG2/R cells was determined using a flow cytometer.

10. Cytocompatibility assay

The cytotoxicity of nanoparticles was evaluated via the MTT assay. Briefly, NIH3T3 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% nonessential amino acid in 5% CO_2/95% air at 37 °C. HepG2/R cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in 5% CO_2/95% air at 37 °C. Cells were seeded in a 96-well plate at a density of 10^4 cells per well. The medium in the wells was replaced with free pCA/pLB Np3 and DOX-loaded pCA/pLB Np3 at different concentrations. After 24 h of incubation, 10 μL of MTT solution was added into each well and the mixture was incubated for another 4 h. The medium was removed, and 150 μL of DMSO was added into each well to dissolve formazane crystals. The optical density was read on a microplate reader at 492 nm in triplicate. Relative cell viability was calculated as a percentage compared with that of untreated control.

11. *In vitro* cellular internalization.

To evaluate receptor-mediated endocytosis, HepG2/R and NIH3T3 cells, respectively, were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated overnight. Then the cells were treated with predetermined concentrations of pCA/pLB Np3 and DOX-loaded pCA/pLB Np3, respectively. After incubation for a predetermined time, cells were washed three times with PBS buffer (pH 7.4), fixed with 4% paraformaldehyde for 10 min and then sequentially stained with DAPI for 10 min. The internalization was determined by CLSM. To investigate the influences of pH values on the cellular uptake of pCA/pLB Np3 by HepG2/R cells, the intracellular distribution was analyzed using CLSM imaging. The cells were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated overnight. Then the cells were incubated with DOX-loaded pCA/pLB Np3 at pH 6.5 or 7.4 in medium without serum, respectively. After incubation for 2 h at 37 °C, the cells were washed with PBS buffer (pH 7.4) and the medium was replaced with fresh complete medium. After being incubation for 6 h at 37 °C, the cells were washed three times with PBS buffer (pH 7.4), fixed with 4% paraformaldehyde for 10 min, and washed three times with PBS, stained with DAPI for 10 min and washed three times with PBS buffer (pH 7.4) before the observation using CLSM.

Flow cytometry was performed to quantitatively investigate the pH-sensitive cellular uptake and receptor-mediated endocytosis of pCA/pLB nanoassemblies. For pH-sensitive cellular uptake, the cells were seeded in 12-well plates at a density of 1×10^5 cells/well overnight, and then incubated with DOX-loaded pCA/pLB Np3 in medium without serum for 4 h at pH 6.5 and 7.4, respectively. Then, cells were washed, trypsinized, collected and resuspended in 1 mL 4% paraformaldehyde for analysis using a flow cytometer. For competition inhibition

assay of pCA/pLB Np3 uptake by HepG2/R cells, the cells were treated with free galactose or PBA-NH_2 at predetermined concentration for 1 h before incubated with pCA/pLB Np3 for 4 h at 37 °C. Then, cells were washed, trypsinized, collected and resuspended in 1 mL 4% paraformaldehyde for flow cytometric analyses.

12. *In vivo* administration

12.1. Animals

Male Balb/c nude mice (4-week-old) were purchased from Beijing HFK Bioscience Co., Ltd (Beijing, China), and all the protocols for animal experiments were carried out according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Animal Care and Use Committee of Nankai University.

12.2. Plasma clearance

The pharmacokinetics of nanoparticles was investigated with SD mice (n = 5) through intravenous administration of pCA/pLB Np3 (0.2 mL, 4 mg/mL). At predetermined time points, blood samples were collected from the retro-orbital plexus of the mouse eye, placed in heparinized tubes and centrifuged to obtain plasma. The fluorescence intensity of nanoparticles in the plasma was determined using the fluorescence spectrophotometer (RF-5301PC).

12.3. Tumor xenograft model

The HepG2 xenograft tumor model was established by subcutaneous injection of 1×10^6 cells (0.1 mL cell suspension) into the right flanks of Balb/c nude mice. The tumor volume (mm^3) was calculated by the following equation: volume = $ab^2/2$, where a and b were width and length of the tumor, respectively.

12.4. Tumor suppression study

Nude mice were randomly divided into three groups (five mice per groups). When the tumor volume of mice reached a mean size about 50 mm^3, PBS buffer (pH 7.4), free DOX and DOX-loaded pCA/pLB Np3 were intravenous injected into mice on the 21st, 26th and 30th day, respectively, at an equivalent dose of 1 mg DOX per kg of mouse body weight. The tumor size and body weight were measured at a predetermined time.

12.5. Histological analysis

On the 40th day, major organs of mice treated with PBS buffer (pH 7.4), free DOX and DOX-loaded nanoparticles were harvested and fixed in 10% neutral buffered formalin for histochemical studies. They were embedded in paraffin, and 4 μm thick sections were cut and stained with hematoxylin and eosin. Micrographs were obtained using an optical microscope.

13. *In vivo* imaging

13.1. *In vivo* imaging and biodistribution analysis

For *in vivo* fluorescence imaging, pCA/pLB Np3 were injected intravenously in HepG2/R xenograft tumor model at a concentration of 2.0 mg/kg body weight. Then, under isoflurane anesthesia, the mice were imaged using an IVIS Lumina II *in vivo* imaging system at different time points post-injection.

After fluorescence imaging 24 h post-injection, mice were sacrificed to collect organs (including heart, liver, spleen, lung and kidneys) and tumors for ex vivo imaging and biodistribution analysis. Images were obtained using the *ex vivo/in vivo* imaging system (Xenogen, IVIS Lumina II, US) with a 465 nm excitation wavelength and a 570 nm filter to collect the fluorescence signals of nanoparticles. Fluorescence intensity was measured by

drawing regions of interest (ROIs) over removed NPs. Quantitative data were expressed as means ± SD.

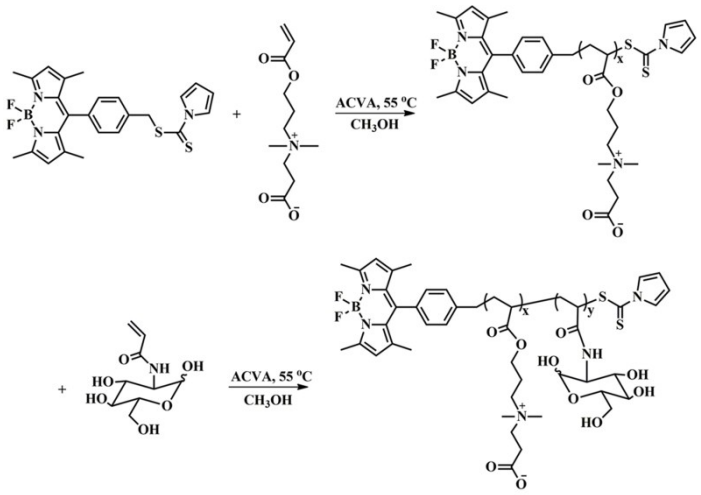
13.2. In situ real-time tracing nanocarriers in tumor 3D imaging

To explore the trace of this nanoassembly inside the tumor in spatial level, pharmacokinetic-DFT was used to in situ real-time measure the ICG imaging of naked ICG and ICG-loaded pCA/pLB Np3. The pharmacokinetic-DFT system was designed by Zhang *et. al*.[6] and the pharmacokinectic imaging assay was first performed using the Balb/c nude mice bearing liver tumor. For *in vivo* pharmacokinectic-DFT imaging, 400 μL of ICG (50 μg/mL) and ICG-loaded pCA/pLB Np3 (the content of ICG was 50 μg/mL) were injected intravenously into the mice (n = 3), then the *in vivo* pharmacokinectic imaging was performed under DFT system at 5 min, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-injection, respectively.

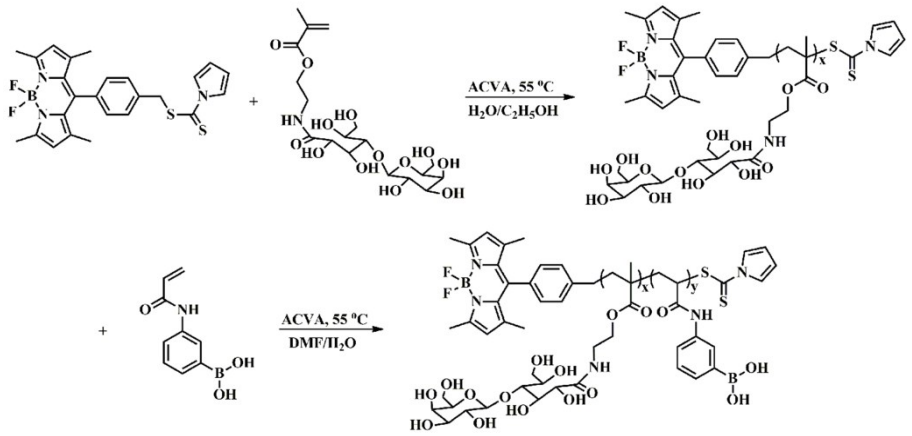
14. Statistical analysis

Data from different experimental groups were compared with the control group by a one-way analysis of Kruskal-Wallis ANOVA. The level of statistical significance was set at $p < 0.05$.

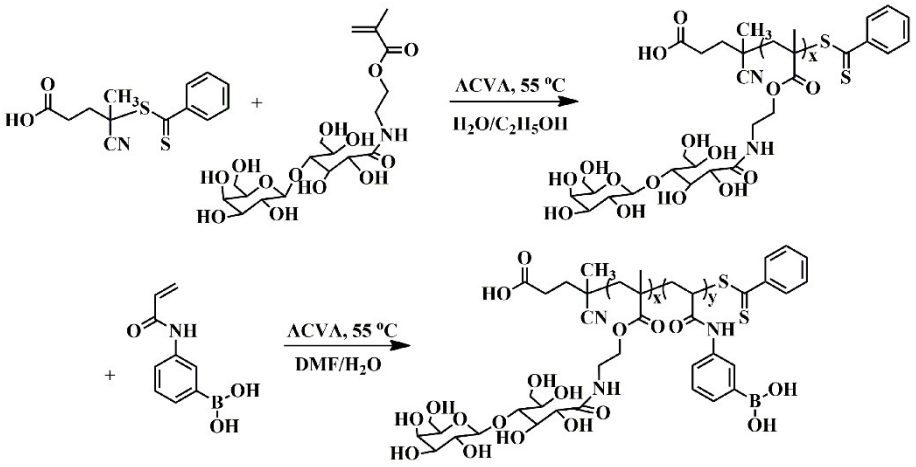
(A) Synthesis of pCBAA-*b*-pAGA



(B) Synthesis of pLAMA-*b*-pAAPBA



Scheme S1. Synthetic routes of (A) pCBAA-*b*-pAGA and (B) pLAMA-*b*-pAAPBA copolymers.



Scheme S2. Synthetic route of non-fluorescent pLAMA-*b*-pAAPBA copolymer with CPADB by RAFT polymerization.

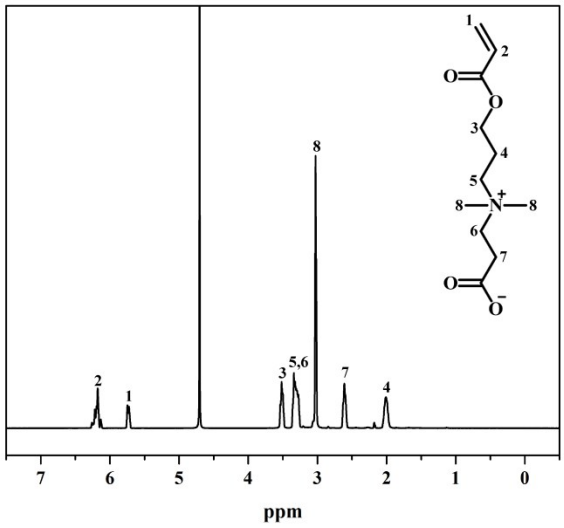


Figure S1.[1]H NMR spectrum of CBAA (D_2O).

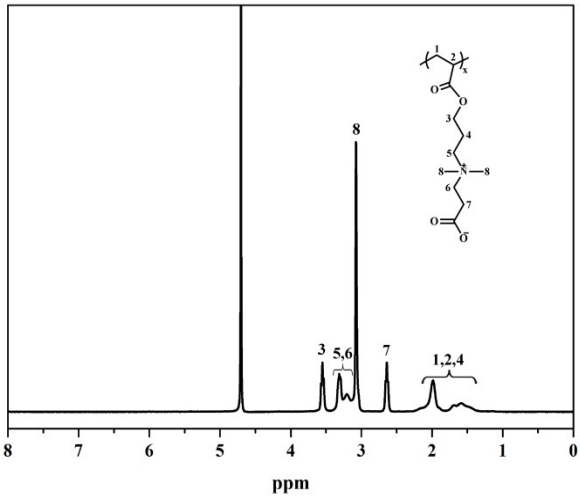


Figure S2.[1]H NMR spectrum of pCBAA (D_2O).

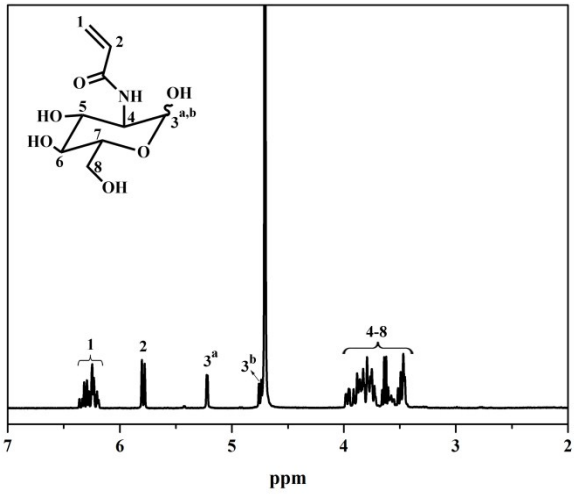


Figure S3.[1]H NMR spectrum of AGA (D_2O).

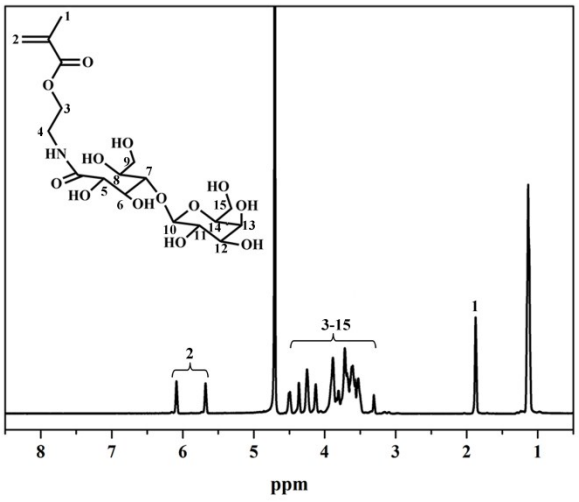


Figure S4. ^{1}H NMR spectrum of LAMA (D_2O).

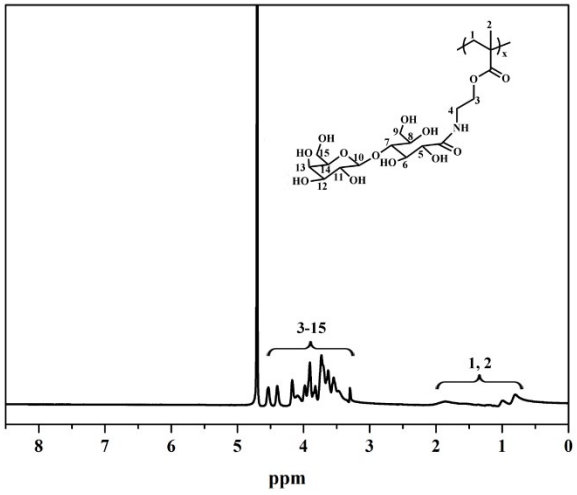


Figure S5. ^{1}H NMR spectrum of pLAMA (D_2O).

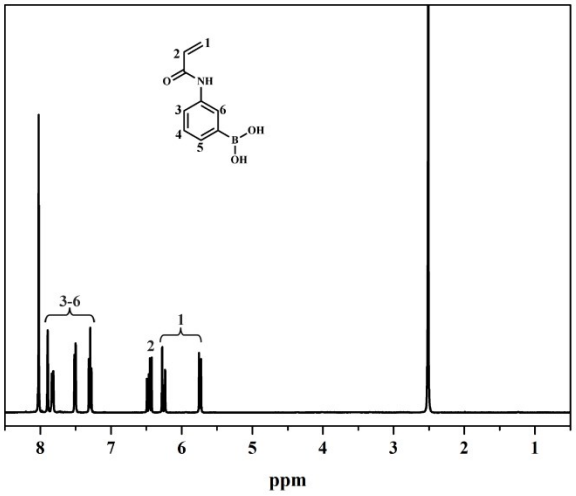


Figure S6.^{1}H NMR spectrum of AAPBA (DMSO-d_6).

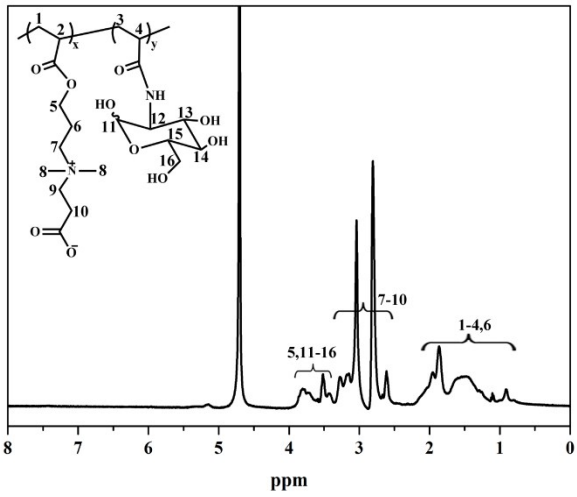


Figure S7. ^{1}H NMR spectrum of copolymer pCBAA-*b*-pAGA (D_2O)

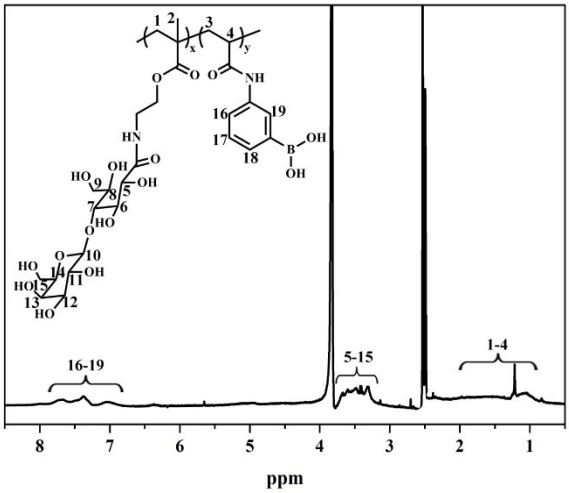


Figure S8. ^{1}H NMR spectrum of copolymer pLAMA-*b*-pAAPBA (DMSO-d_6/D_2O, v/v = 1:1).

Table S1. Constitution of pCBAA-*b*-pAGA and pLAMA-*b*-pAAPBA copolymers.

Sample	Monomer		CBAA/AGA		LAMA/AAPBA	
			Theory[a]	^{1}H NMR[b]	Theory[a]	^{1}H NMR[b]
pCBAA$_{30}$-*b*-pAGA$_{10}$	CBAA	AGA	3	3.47	---	---
pLAMA$_{30}$-*b*-pAAPBA$_{10}$	LAMA	AAPBA	---	---	3	2.64
pLAMA$_{30}$-*b*-pAAPBA$_{15}$	LAMA	AAPBA	---	---	2	1.68
pLAMA$_{30}$-*b*- AAPBA$_{20}$	LAMA	AAPBA	---	---	1.5	1.13

[a] The theoretical molar ratio of CBAA/AGA or LAMA/AAPBA, respectively.

[b] The two copolymer compositions were calculated using the ^{1}H NMR integral intensity of signals between the 12H in CBAA moiety and 7H in sugar moiety of AGA, and 4H in phenyl moiety and 12H in sugar moiety of LAMA, respectively.

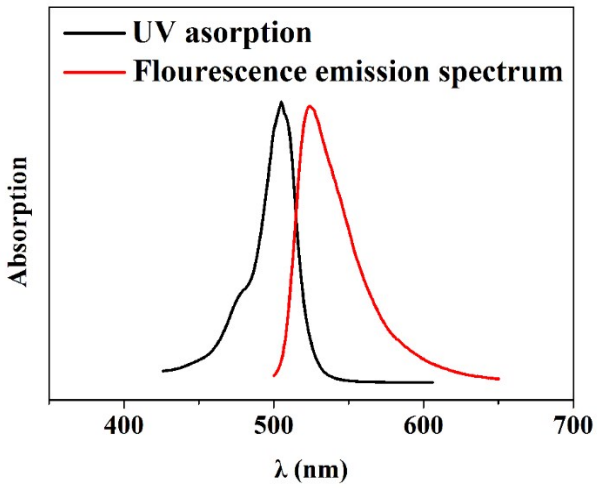


Figure S9. UV absorption and fluorescence emission spectra of pCA/pLB Np3.

Table S2. Characterization and properties of pCA/pLB nanoparticles.

Samples [a]	D_H [b] (nm)	PDI [b]	Zeta potential [b] (mV)
pCA/pLB Np1	117.9 ± 2.4	0.12 ± 0.02	-1.3 ± 0.2
pCA/pLB Np2	151.0 ± 3.6	0.15 ± 0.01	-2.5 ± 0.7
pCA/pLB Np3	165.6 ± 3.1	0.08 ± 0.05	-3.1 ± 1.3

[a] $pCBAA_{30}$-*b*-$pAGA_{10}$/$pLAMA_{30}$-*b*-$pAAPBA_{10}$, $pCBAA_{30}$-*b*-$pAGA_{10}$/$pLAMA_{30}$-*b*-$pAAPBA_{15}$ and $pCBAA_{30}$-*b*-$pAGA_{10}$/$pLAMA_{30}$-*b*-$pAAPBA_{20}$ were tabbed as pCA/pLB Np1, pCA/pLB Np2, and pCA/pLB Np3, respectively.

[b] Hydrodynamic parameter, polydispersity index and zeta potential of nanoparticles in pH 7.4 PBS were measured by DLS at 25 °C.

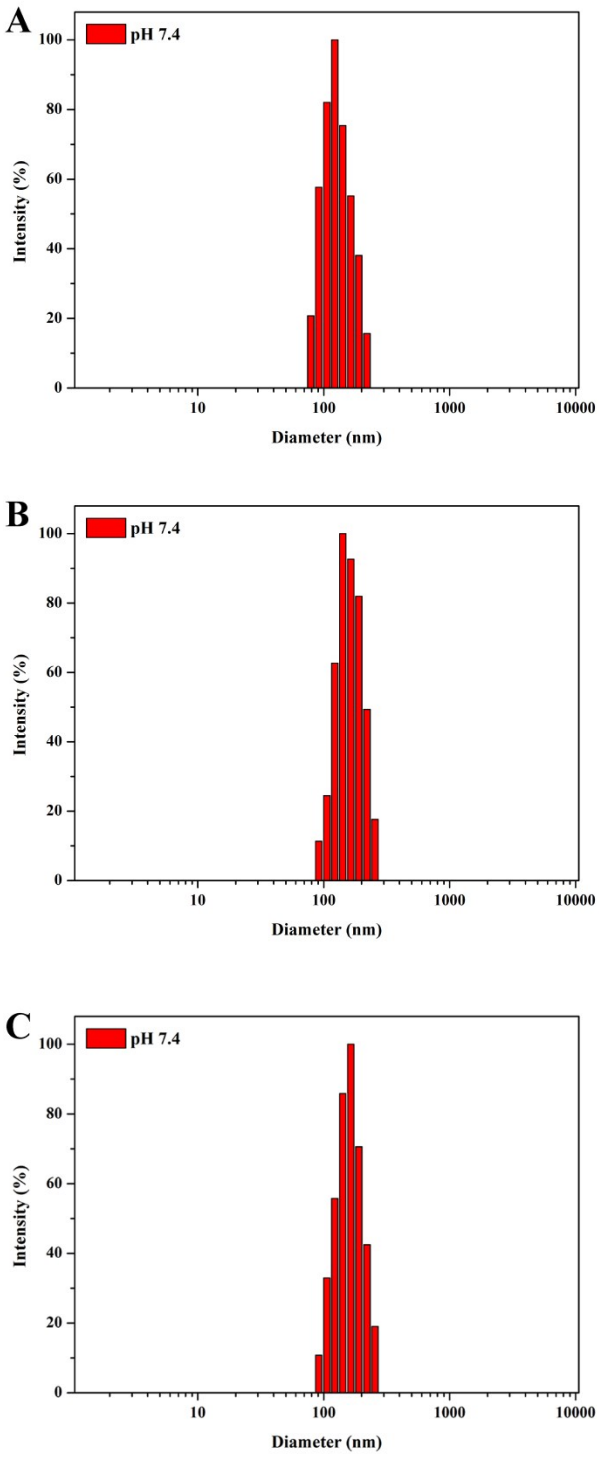


Figure S10. The DLS histograms of (A) pCA/pLB Np1, (B) pCA/pLB Np2 and (C) pCA/pLB Np3 in pH 7.4 PBS buffer.

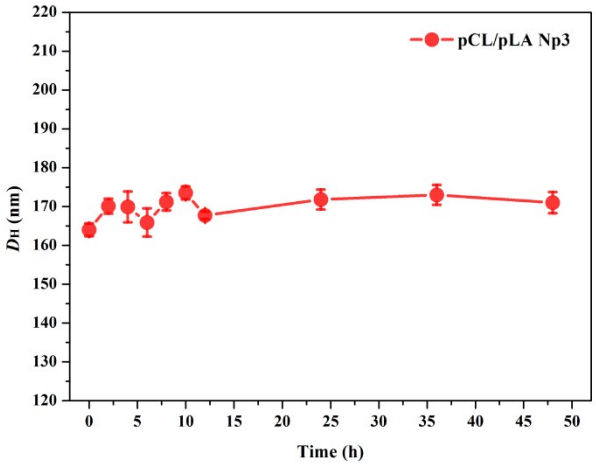


Figure S11. The stability of pCA/pLB Np3 following incubating with 90% fetal bovine serum.

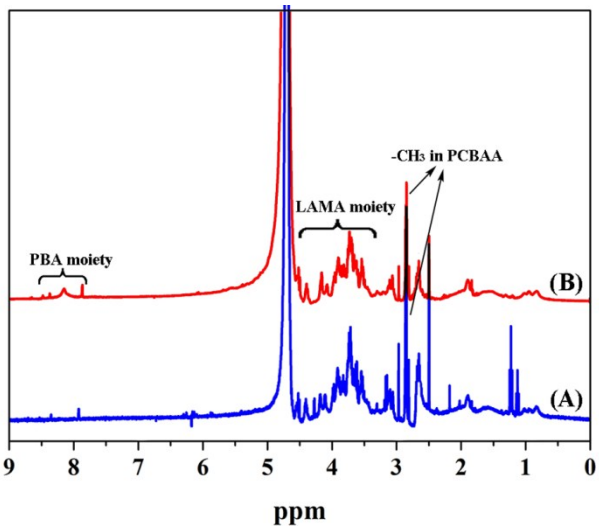


Figure S12. ^{1}H NMR spectra of (A) pCA/pLB Np3 in pH 7.4 solution (D_2O) and (B) shell-detached pCA/pLB Np3 in pH 6.5 solution (D_2O).

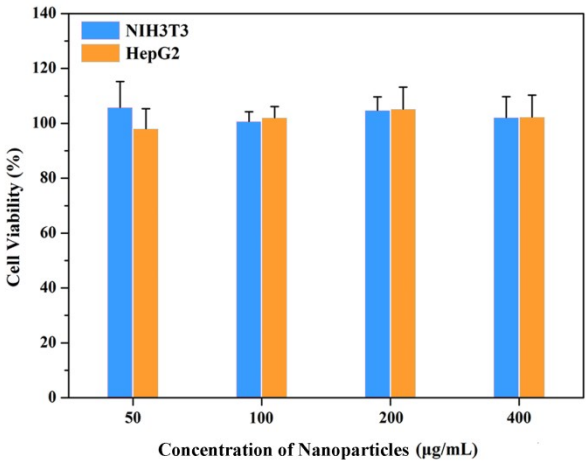


Figure S13. Cell viability of NIH3T3 and HepG2/R cells after incubation with various concentrations of pCA/pLB Np3.

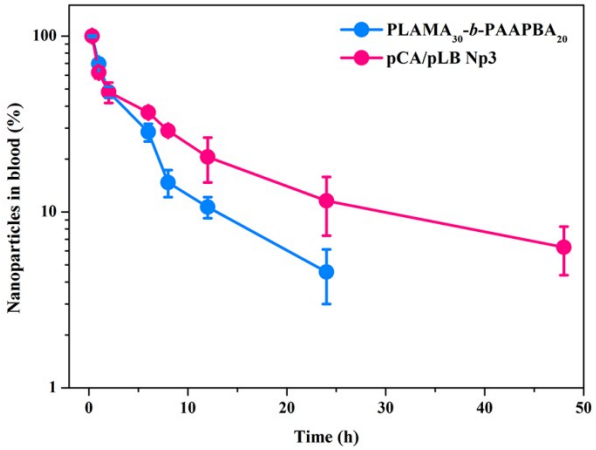


Figure S14. Pharmacokinetic analysis of intravenously administrated pLAMA$_{30}$-*b*-pAAPBA$_{20}$ and pCA/pLB Np3.

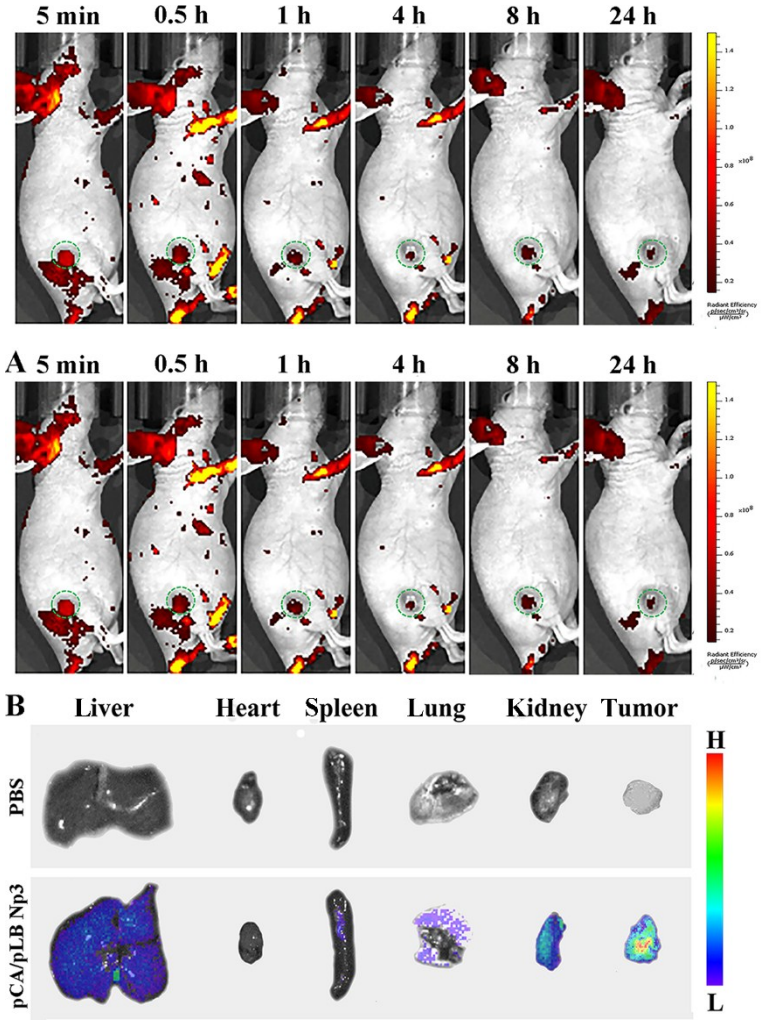


Figure S15. (A) *In vivo* fluorescence imaging of the mice bearing HepG2/ADR tumor injected with BODIPY-labeled pCA/pLB Np3 at the dose of 1.5 mg kg^{-1}. (B) *Ex vivo* imaging and biodistribution of nude mice bearing HepG2/ADR tumors after intravenous injection of free PBS and pCA/pLB Np3.

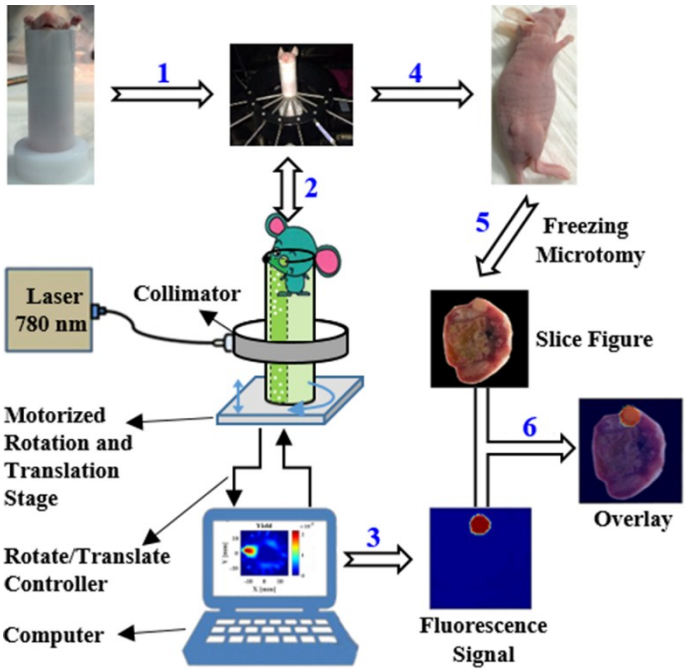


Figure S16. The process of pharmacokinetic-DFT imaging system of the Balb/c nude mice bearing HepG2/ADR tumor: (1) loading mice into DFT system, (2) diagram of DFT system, (3) pharmacokinetic-DFT imaging, (4) removing the mice from DFT system, (5) freezing microtomy, and (6) the overlay of slice figure and fluorescence signals.

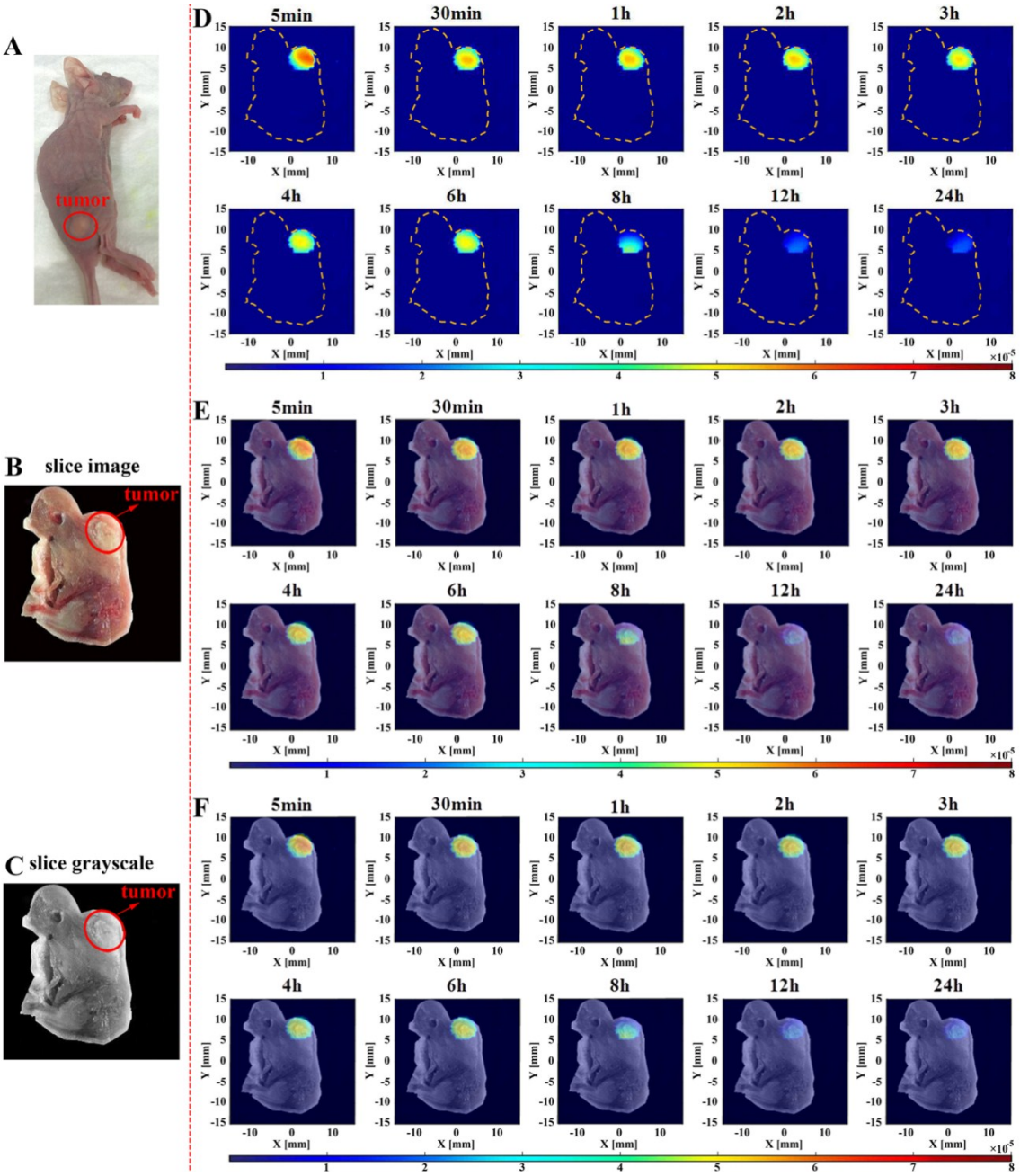


Figure S17. *In vivo* pharmacokinetic imaging under DFT system of the Balb/c nude mice bearing HepG2/ADR tumor injected with ICG over 24 h: (A) the mice photograph, (B) slice image, (C) slice grayscale image, (D) DFT images, (E) the overlay of DFT and slice images, and (F) the overlay of DFT and slice grayscale images.

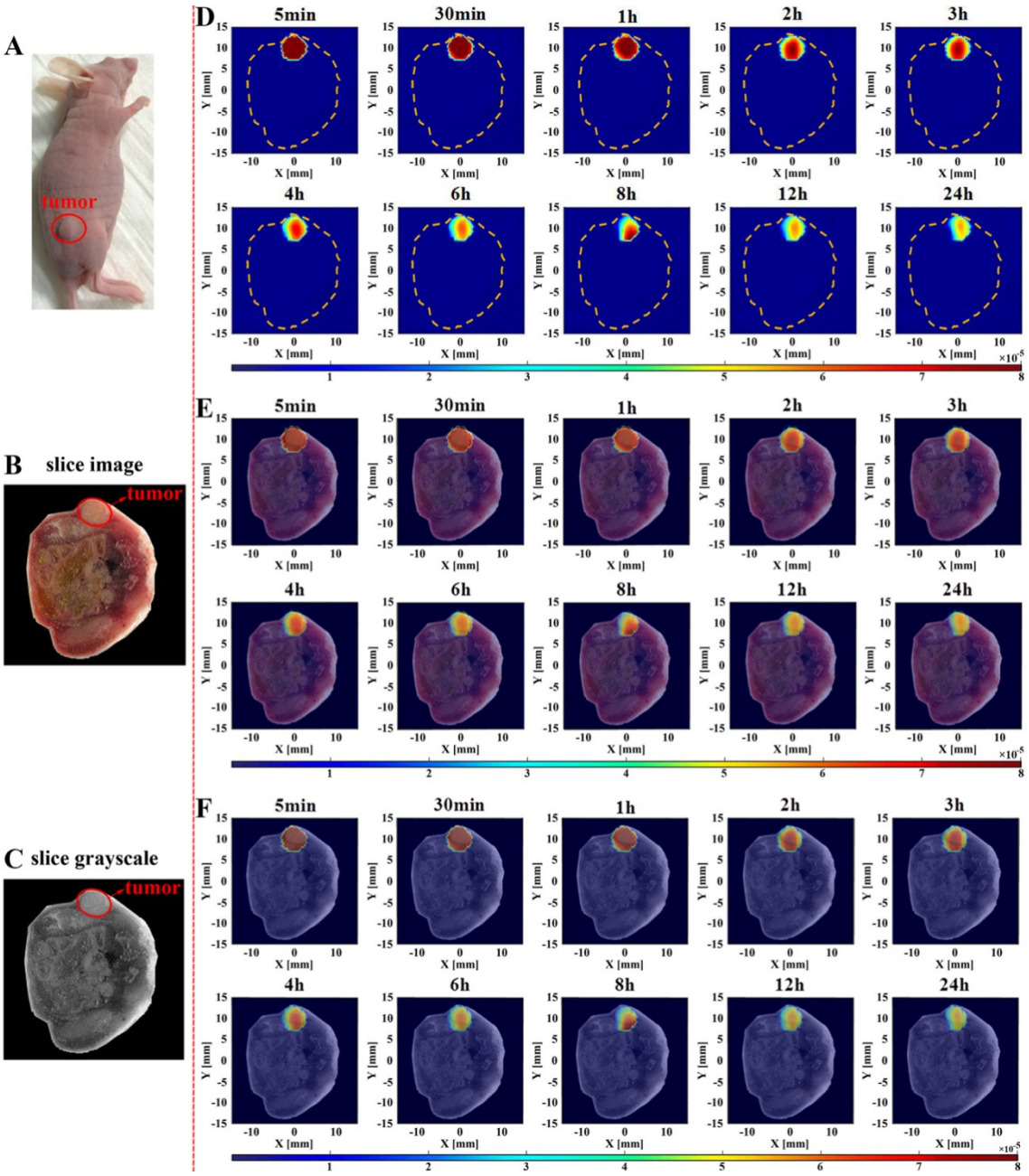


Figure S18. *In vivo* pharmacokinetic imaging under DFT system of the Balb/c nude mice bearing HepG2/ADR tumor injected with ICG-loaded pCA/pLB Np3 over 24 h: (A) the mice photograph, (B) slice image, (C) slice grayscale image, (D) DFT images, (E) the overlay of DFT and slice images, and (F) the overlay of DFT and slice grayscale images.

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