Supporting Information

Liposome-Coated Persistent Luminescence Nanoparticles as Luminescence Trackable Drug Carrier for Chemotherapy

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Supplementary Methods

Chemicals and Materials. 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and cholesterol were purchased from Avanti Polar Lipids (Alabama, USA). Paclitaxel was obtained from MCE China (Shanghai, China). Other reagents were all bought from Aladdin (Shanghai, China).

Characterization. Transmission electron microscopy (TEM) images were acquired on a Talos F200C transmission electron microscope (FEI, USA) in room temperature. Hydrodynamic size distribution and Zeta potential were recorded on a Nano-ZS Zetasizer (Malvern, U.K.). Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA). X-ray diffraction (XRD) experiments were performed on a D/max-2500 diffractometer with Cu Kα radiation (Rigaku, Japan). Luminescence spectra were acquired on an F-4500 spectrofluorometer (Hitachi, Japan). Cell imaging experiments were carried out on a TCS SP8 confocal laser scanning microscope (Leica, Germany). NIR persistence photographs were collected on the IVIS Imaging System (PerkinElmer, USA). The concentration of Zn from organs and feces of treated mice was detected on an X7 inductively coupled plasma mass spectrometer (Thermo Electron, USA).

Preparation of Lipo-PLNP. $Zn_{1.1}Ga_{1.8}Ge_{0.1}O_4:Cr^{3+}$ PLNP was synthesized using a hydrothermal method at 220 °C for 16 h without further calcination. The compositions and details of synthesis were according to our previous work.[1] Liposomes were prepared according to Molinaro et al.[2] Briefly, 10.85 mg of DPPC,

DSPC, DOPC, and cholesterol (5:1:3:1 molar ratio) were dissolved in chloroform: methanol (3:1 v/v) mixture. Then, the solvent was evaporated using a rotary evaporator to form a thin film and completely dried through a vacuum pump. The dried films were hydrated with PLNP dispersion (5 mg/mL, 5 mL) or water alone, subsequently with three cycles of heating at 45 °C and ultrasonic bathing (3 min each). Then the suspensions were extruded eleven times through 200 nm pore-size cellulose acetate membranes to assemble Lipo-PLNP and liposomes, respectively. The non-encapsulated PLNPs were removed by centrifugation at 2000 rpm for 10 min.

PTX Encapsulation and Release Properties of Vesicles. To assemble Lipo-PTX or Lipo-PLNP-PTX, paclitaxel (0.45 mg) was firstly dissolved in the chloroform/methanol mixture. The other steps were the same as those for the preparation of Lipo-PLNP. The quantification of PTX loaded into liposomes or Lipo-PLNP was analyzed by HPLC (Waters, USA). Two milliliters of Lipo-PTX or Lipo-PLNP-PTX were diluted with 4 mL methanol and treated under ultrasonic bath. Another 2 mL of Lipo-PTX or Lipo-PLNP-PTX was centrifuged at 2000 rpm for 10 min. The precipitate was dissolved in 1 mL of methanol. To study the release properties of PTX, freshly prepared Lipo-PTX or Lipo-PLNP-PTX suspension were mixed with different media in different ratio (suspension: 20 mM PBS = 1:1; suspension: 100% human serum = 1:1, 1:3; suspension: whole blood = 1:3). Finally, twenty microliters of processed samples were injected into a C18 guard column (methanol: water, 80: 20) after filtration through organic membrane. The column temperature was 40 °C. The flow rate was 1.0 mL min^{-1} and the detection wavelength

was set at 227 nm. The encapsulation efficiency of PTX was calculated according to equation 1:

$$\text{Encapsulation efficiency (\%)} = (PTX_{total} - PTX_{free}) / PTX_{total} \times 100\% \quad (1)$$

The release properties of Lipo-PTX and Lipo-PLNP-PTX were evaluated under storage condition at 4 °C in PBS (10 mM, pH 7.4) or in 50% human serum at 37 °C. Two hundred microliters of samples were taken out in different time. Other procedures followed those for determining the encapsulation efficiency of PTX.

Cell Culture. MCF-7 cells were cultured in DMEM medium with 10% FBS and 1% penicillin-streptomycin in 5% CO_2 at 37 °C. Cells were adhered in confocal dish overnight and incubated with Lipo-PLNP for 12 h. The cells were washed with PBS and fixed, stained with DAPI before cell imaging.

In Vitro Cytotoxicity Assay. MCF-7 or 293T cells (1×10^4 cells/well) were cultured in DMEM or RPMI 1640 medium, respectively. Cells were incubated with different concentrations of Lipo-PLNP for 24 h to evaluate the cytotoxicity. The cell viability was analyzed by MTT assay.

To test the cytotoxicity of Lipo-PLNP-PTX, MCF-7 cells were seed in 96-well plates (1×10^4 cells/well) and incubated with different concentrations of Lipo-PLNP-PTX for 24 h. The cell viability was also obtained by subsequent MTT assay.

In Vivo Luminescence Imaging of MCF-7 Tumor. Balb/c athymic nude mice (5 weeks old, female) were subcutaneously injected with MCF-7 cells (5×10^6

cells/mouse) to produce tumors 5-7 mm in size for optical imaging. After 254 nm UV light excitation for 10 min, Lipo-PLNP (200 μL, 2.3 mg mL^{-1} as PLNP) was intravenously injected into anesthetized tumor-bearing mice. A LED light (650 ± 10 nm, 5000 lm) was excited on the mice for 2 min before acquiring optical images at different times. Animal experiments followed the rules of the Tianjin Committee of Use and Care of Laboratory Animals.

In Vivo Therapeutic Effect. Balb/c nude mice bearing a MCF-7 tumor (tumor diameter ~5-7 mm) were randomly divided into three groups (n = 8). Lipo-PLNP-PTX (200 μL, 2.3 mg/mL as PLNP, 63 μg/mL as PTX) was intravenously injected into the mice in experiment group once every two days for three times. Other two groups were injected into PBS and Lipo-PLNP as control. The tumor length and width were measured using a digital caliper every two days for two weeks after the first injection. The volumes were calculated based on length × $width^2/2$. Three mice of each group were sacrificed at two days after injection. The tumor tissue was performed for histology study.

Histological Staining. Three groups of healthy mice (n = 3) were intravenously injected with Lipo-PLNP (200 μL, 4.6 mg/mL as PLNP) and PBS only (200 μL) once every two days for three times. The mice were sacrificed after 14 days and the major organs were collected. The organs were fixed in 4% formaldehyde for 24 h and embedded into paraffin. Finally, the sections of 10 μm thickness were made for hematoxylin-eosin (H&E) staining.

Supplementary Figures

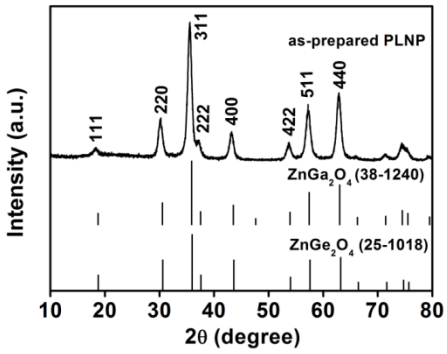

Figure S1. XRD pattern of the as-synthesized PLNP.

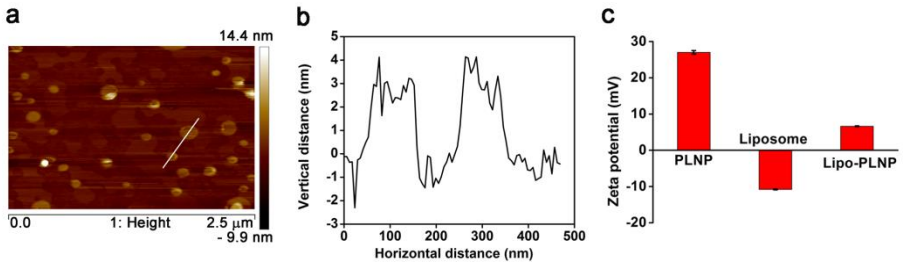

Figure S2. (a) AFM image of the Lipo-PLNP dropped on silicon slice. (b) The corresponding height profile of the AFM image. (c) Zeta potential of the PLNP, liposome, and Lipo-PLNP.

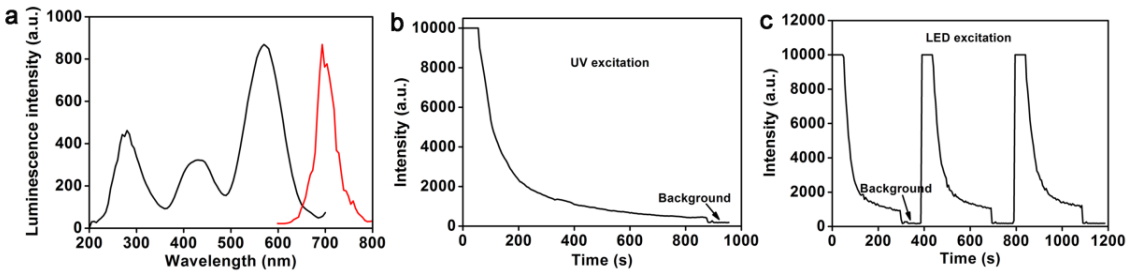

Figure S3. (a) Excitation and emission spectra of the PLNP powder (Ex slit: 5 nm, Em slit: 10 nm, PMT voltage: 700 V). (b) Persistent luminescence decay curve of the PLNP powder monitored at 695 nm after 5 min illumination with a UV lamp (Ex slit: 10 nm, Em slit: 20 nm, PMT voltage: 950 V). (c) Persistent luminescence decay curve of the PLNP powder monitored at 695 nm repeatedly activated by a 650 nm LED light (Ex slit: 10 nm, Em slit: 20 nm, PMT voltage: 950 V).

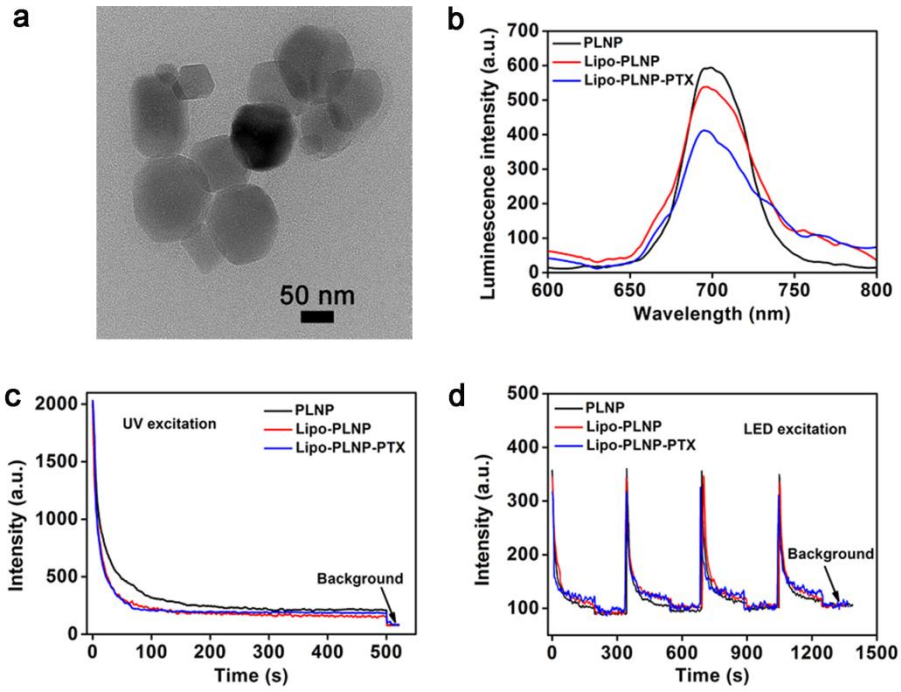

Figure S4. (a) TEM image of the Lipo-PLNP-PTX. (b) Emission spectra of the PLNP, Lipo-PLNP and Lipo-PLNP-PTX dispersions with the same concentration of PLNP (Ex slit: 10 nm, Em slit: 10 nm, PMT voltage: 700 V). (c) Persistent luminescence decay curves of the PLNP, Lipo-PLNP and Lipo-PLNP-PTX dispersions monitored at 695 nm after 5 min irradiation with a UV lamp (Ex slit: 10 nm, Em slit: 20 nm, PMT voltage: 950 V). (d) Persistent luminescence decay curves of the PLNP, Lipo-PLNP and Lipo-PLNP-PTX dispersions monitored at 695 nm repeatedly activated by a 650 nm LED light (Ex slit: 10 nm, Em slit: 20 nm, PMT voltage: 950 V).

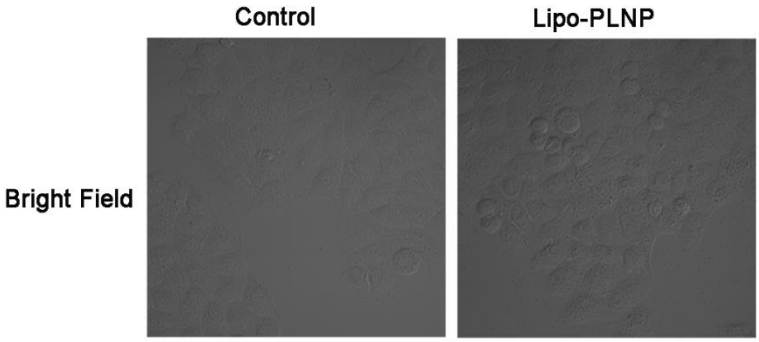

Figure S5. Bright field images of MCF-7 cells treated with medium in the absence or presence of Lipo-PLNP.

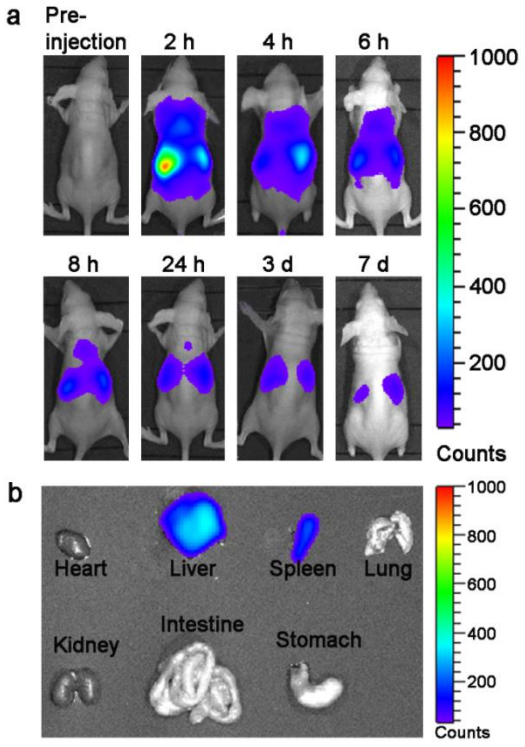

Figure S6. (a) In vivo luminescence imaging with the Lipo-PLNP after intravenous injection in normal mice for 7 days (10 min illumination with a 254 nm UV light before intravenous injection, 2 min excitation with LED lamp before each acquisition). (b) Persistent luminescence images of the major organs dissected from sacrificed animal at 7 days post-injection.

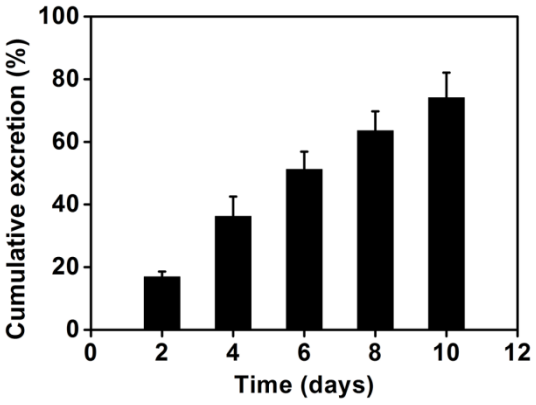

Figure S7. The cumulative excretion of Lipo-PLNP with time via feces of mice.

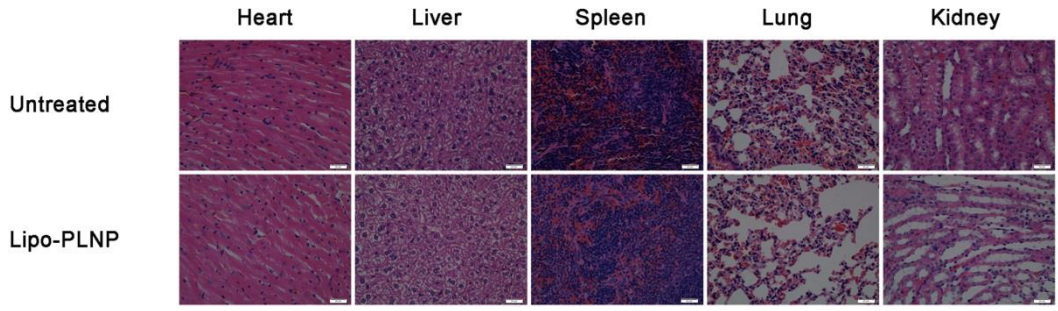

Figure S8. Histological staining of major organs from the Lipo-PLNP treated mice and normal mice. Scale bar: 20 μm.

Supplementary References

(1) Chen, L. J.; Sun, S. K.; Wang, Y.; Yang, C. X.; Wu, S. Q.; Yan, X. P. *ACS Appl. Mater. Interfaces* **2016**, *8*, 32667-32674.

(2) Molinaro, R.; Corbo, C.; Martinez, J. O.; Taraballi, F.; Evangelopoulos, M.; Minardi, S.; Yazdi, I. K.; Zhao, P.; De Rosa, E.; Sherman, M. B.; De Vita, A.; Toledano Furman, N. E.; Wang, X.; Parodi, A.; Tasciotti, E. *Nat. Mater.* **2016**, *15*, 1037-1046.