Supplementary Information

Ultrabright and Multicolorful Fluorescence of Amphiphilic Polyethyleneimine Polymer Dots for Efficiently Combined Imaging and Therapy

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Materials and methods

Materials and cell lines

Branched polyethyleneimine (PEI, 25kD) was obtained from Sigma-Aldrich (St. Louis, MO, USA). D,L-Lactide (DLLA), was obtained from Alfa Aesar (Ward Hill, MA, USA). Paclitaxel (PTX) was purchased from NuoRi Co, Ltd. (Beijing, China). CCK-8 Kits were purchased from Dojindo Molecular Technologies (Japan). Unless specified, all of the commercial products were used without further purification.

Human breast adenocarcinoma (MCF-7) cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin (100 U/mL) and streptomycin (100 U/mL).

All other reagents and solvents were of analytical grade.

Synthesis and characterization of PEI-PLA copolymers

Pre-dehydrated (15g, 10g and 5g) DLLA and (0.25g) PEI were dissolved in 50mL anhydrous dimethylsulfoxide by stirring, respectively, and then 0.05mol triethylamine was added. The solution was kept at 86°C with constant stirring under nitrogen for 12h. Subsequently, the reacted solution was added to ice-water and the precipitate collected and thoroughly washed with distilled water. Finally, the obtained product was extracted using toluene to remove homopolymer.

The chemical structures of the polymers were characterized with Fourier-transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (^{1}H NMR) spectral analysis. FT-IR spectra of the polymers were detected on a spectrophotometer (Perkin-Elmer, Fremont, CA, USA) using KBr as a reference. ^{1}H NMR spectra of the polymers were obtained with a Bruker AVANCE 400 NMR spectrometer (Billerica, MA, USA). The samples were dissolved in $(CD_3)_2SO$. In addition, the fluorescence spectra were detected using a LS-55 Fluorescence Spectrometer (Perkin-Elmer, Fremont, CA, USA) and the fluorescence quantum yield was determined with quinoline sulfate as the reference.

Preparation of PTX-loaded PDs.

In brief, 20 mg of PEI-PLA copolymers and a given amount of PTX were dissolved in 2mL dichloromethane. The mixture was then slowly added to 10 mL aqueous solution with 1-4% (w/v) of polyvinyl alcohol (PVA) under gentle stirring for 10 min. The emulsion was then sonicated for 5 min at 40W and then evaporated under reduced pressure to remove the dichloromethane. After that, the PDs suspension was centrifuged at 15,000 rpm for 15 min and washed with deionized water three times.

Encapsulation efficiency and loading content of PTX-loaded PDs

The drug encapsulation efficiency and loading content were measured by HPLC (Waters 2478, Milford, MA, USA). A C18-column (Nova-Pak 3.9 × 250 mm, Waters, Milford, MA) was used with a constant mobile phase (water: acetonitrile, 45:55) and the flow rate was set at 1 mL/min. The PTX was extracted with acetonitrile from the lyophilized PTX-loaded PDs and then filtered to get a clear solution. The PTX concentration in the solution was determined at 227 nm with an ultra-violet detector. The loading content and encapsulation efficiency were defined as follows:

Loading content (%) = $W_t / W_s \times 100\%$

Encapsulation efficiency (%) = $W_t / W_0 \times 100\%$

W_0 and W_t are the weight of initial PTX and PTX detected in the PDs. W_s is the weight of PDs after lyophilization.

Characterization of blank PDs and PTX-loaded PDs

Particle size, size distribution and zeta potential were determined by dynamic light scattering (DLS) with a ZetaSizer Nano series Nano-ZS (Malvern Instruments Ltd, Malvern, UK). For the measurement, PDs were appropriately diluted in distilled water, PBS and DMEM (with 10% FBS). The morphology of the PDs was determined by transmission electron microscopy (TEM) (EM-200CX; Jeol Ltd., Tokyo, Japan) under negative staining with uranyl acetate.

***In vitro* drug release**

Typically, 20mg PTX-loaded PDs were dispersed in 5 mL deionized water and then transferred into a dialysis bag (MWCO: 3000Da). The dialysis bag was then

incubated in 35 mL of phosphate buffer solution containing 0.1% (v/v) Tween 80 at pH 7.4 or 5.2 at 37°C and vibrated at a constant rate of 150 rpm. At given intervals, 0.5 mL of the medium was extracted and analyzed for PTX with HPLC as described above. After that, the incubation medium was replaced with the fresh PBS at the same pH. In the assessment of drug release behavior, the cumulative amount of released drug was calculated, and the percentages of drug released from PDs were plotted against time.

***In vitro* and *in vivo* fluorescence imaging.**

For the *in vitro* imaging, MCF-7cells were cultured in DMEM medium containing PDs for 4 h in confocal dishes. The cells were then imaged with a laser confocal scanning microscope (Zeiss) equipped with a 60× oil immersion lens. The excitation wavelengths were 405, 488 and 543nm. To minitor the colocalization between PDs and endosomes/lysosomes, MCF-7cells were stained with lysotracker red (Molecular Probes, USA) after culturing in DMEM medium containing PDs for various times. Then the cells were imaged by confocal microscopy and the data were analysed according to the method described before.

For the *in vivo* imaging, mice were injected subcutaneously and intramuscularly in the right flank with blank PDs respectively and imaged immediately with green excitation (503-548nm) using the Maestro *in vivo* optical imaging system. For systemic administration, blank PDs were intravenously injected into nude mice (20mg/kg). The mice were imaged under anesthesia several different times after injection using the

Maestro *in vivo* optical imaging system. The organs (heart, kidney, liver, lung and spleen), collected at multiple time points after injection, were also imaged.

Cytotoxicity assays

MCF-7 cells were seeded at a density of 5×10^3 cells per well in 96-well plates in DMEM medium and incubated for 24 h. The medium was then replaced with 200 μL of medium containing various equivalent concentrations of blank PDs and PEI; or blank PDs, free-PTX and PTX-loaded PDs. The cells were incubated for 48h and cytotoxicity assays were performed using CCK-8 Kits (Dojindo Molecular Technologies, Tokyo, Japan). Absorbance was detected at 450 nm using a TECAN Infinite M200 microplate reader (Tecan, Durham, USA). All data were presented as mean percentages ± SEM in triplicate compared to the OD values of untreated cells.

***In vivo* therapeutic efficacy**

Female nude mice (18-20g) were purchased from Beijing Vital River Company (Beijing, China) and housed under standard conditions with free access to food and water. All animal experiments were performed in accordance with the principles of care and use of laboratory animals. Next, 2×10^6 MCF-7 cells in 100 μL of physiological saline were injected subcutaneously into the right flank of nude mice. The animals were randomly divided into 3 groups (five mice per group) and when the tumors reached about 100 mm^3 they were treated with saline, free PTX (10 mg/kg) or PTX-loaded PDs(administered at a PTX-equivalent dose of 10 mg/kg). Drugs were administered by intravenous (i.v.) injection every 3 days for a total of seven times. Tumor progression in the mice was then monitored every three days. Tumor volumes

were calculated as (length $\times$ width^2)/2 (mm^3). The mice were sacrificed at the end of the experiment, and their tumors were immediately removed and weighed. All of the animal experiments were conducted under approved protocols of the Institutional Animal Care and Use Committee at the Institute of Tumor in the Chinese Academy of Medical Science.

Statistical analysis

All results are presented as mean ± standard deviation (SD). The t-test was applied to evaluate significant differences among groups according to Bonferroni's post-test. $P<0.05$ was considered to be statistically significant.

Figures and Tables

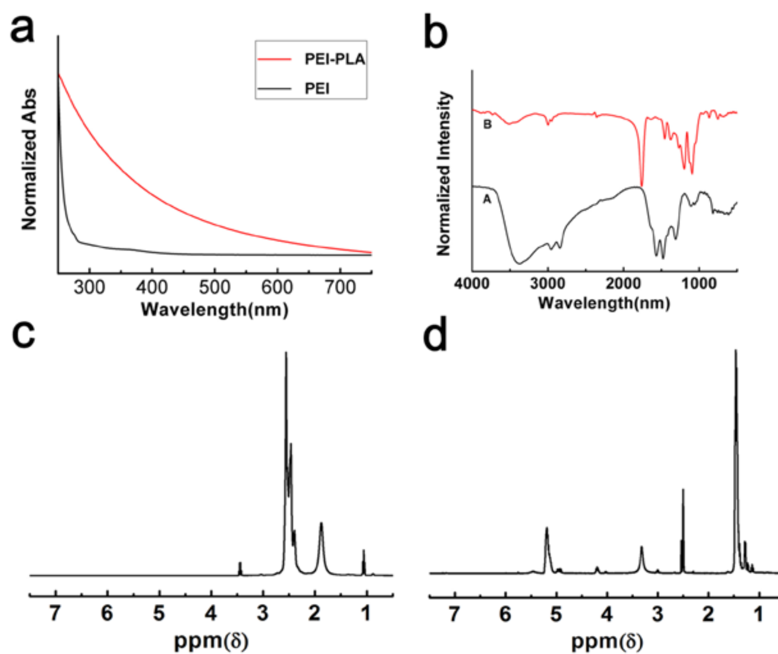


Fig. S1.(a) UV-vis absorption spectra of PEI and PEI-PLA copolymer; (b)FT-IR spectra of PEI (A) and PEI -PLA (B); ^{1}HNMR spectra of PEI (c) and PEI-PLA (d).

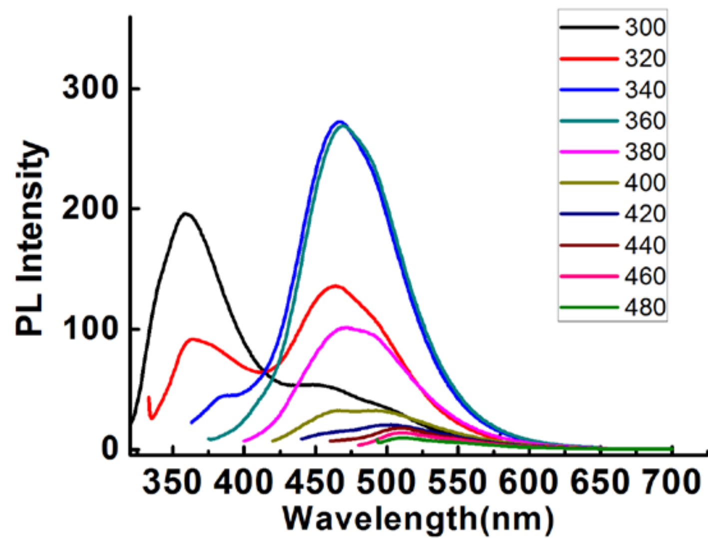


Fig. S2. The luminescence emission spectra (with progressively longer excitation wavelengths in 20 nm increments from 300 nm on the left) of the solution of PEI.

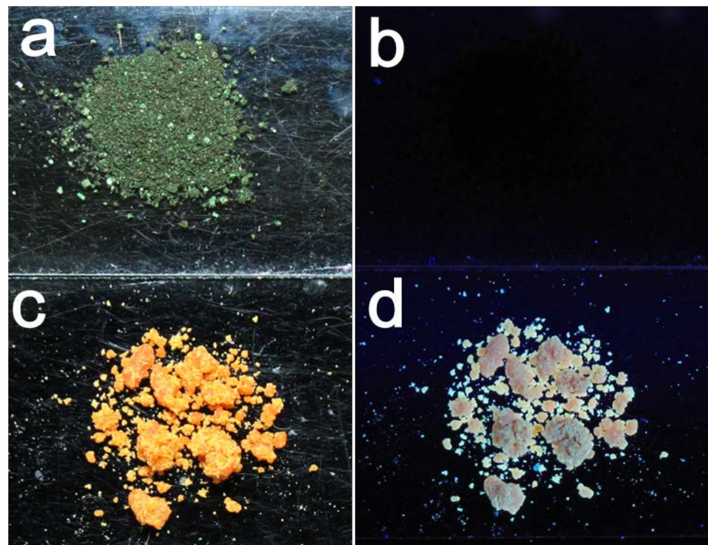


Fig. S3. Photographs of powder formulations of Rhodamine B (a, b) and PEI-PLA copolymer (c, d) taken under normal laboratory lighting (a, c) and UV illumination (360nm) (b, d).

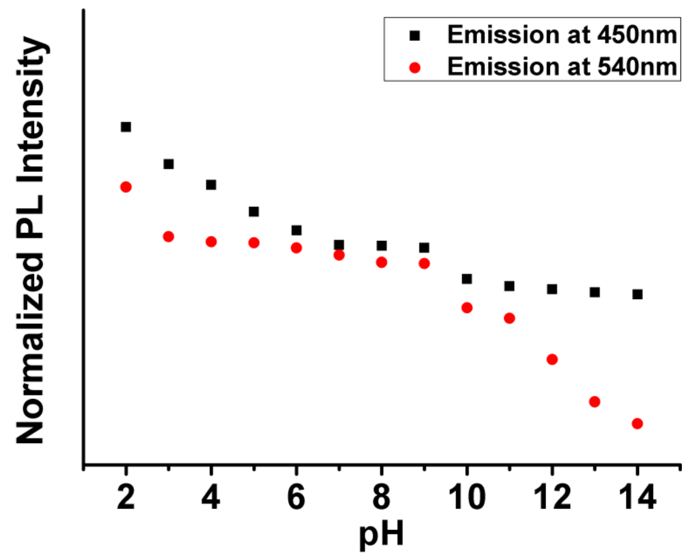


Fig. S4. The fluorescence intensity of the PDs solution with the emission at 450 nm and 540 nm at different pH.

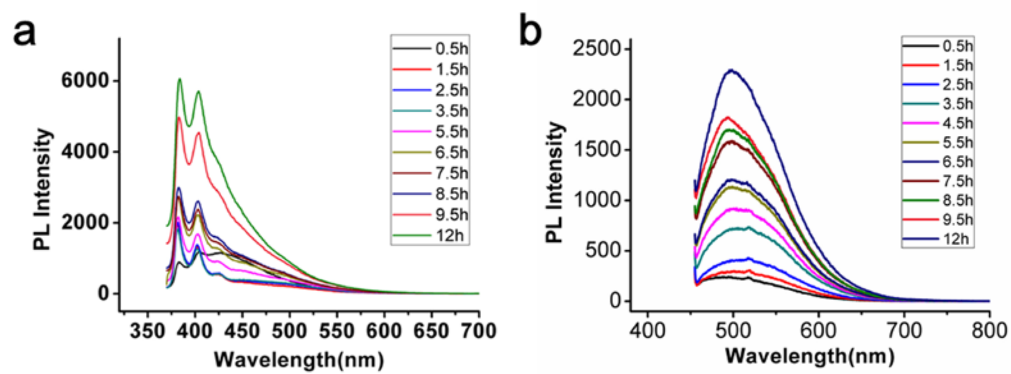


Fig. S5. The fluorescence intensity of the PEI-PLA copolymer were taken out at different interval from the reaction solution when excited at 320 nm (a) and 420 nm (b).

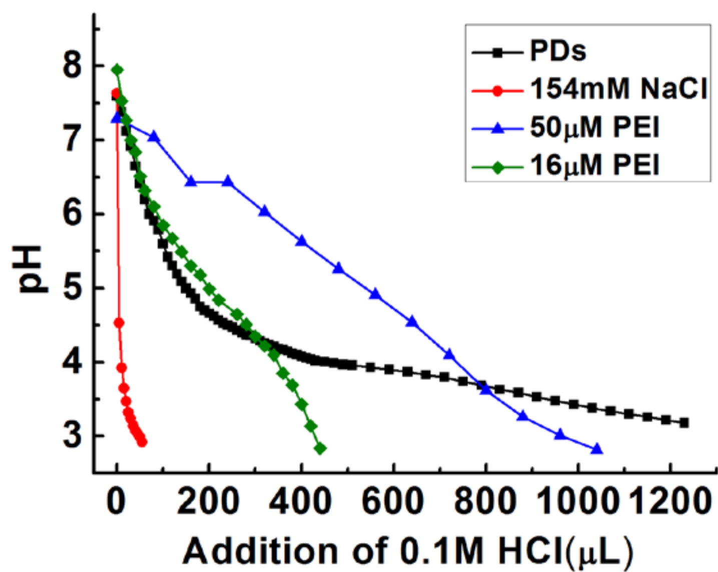


Fig. S6. Proton-buffering capacity of PDs in solution (10 mg/mL; 116 mmol of amines in 10 mL), 16 μM PEI (94 μmol of amines in 10 mL), 50 μM PEI (290 μmol of amines in 10 mL) and NaCl (154 mM).

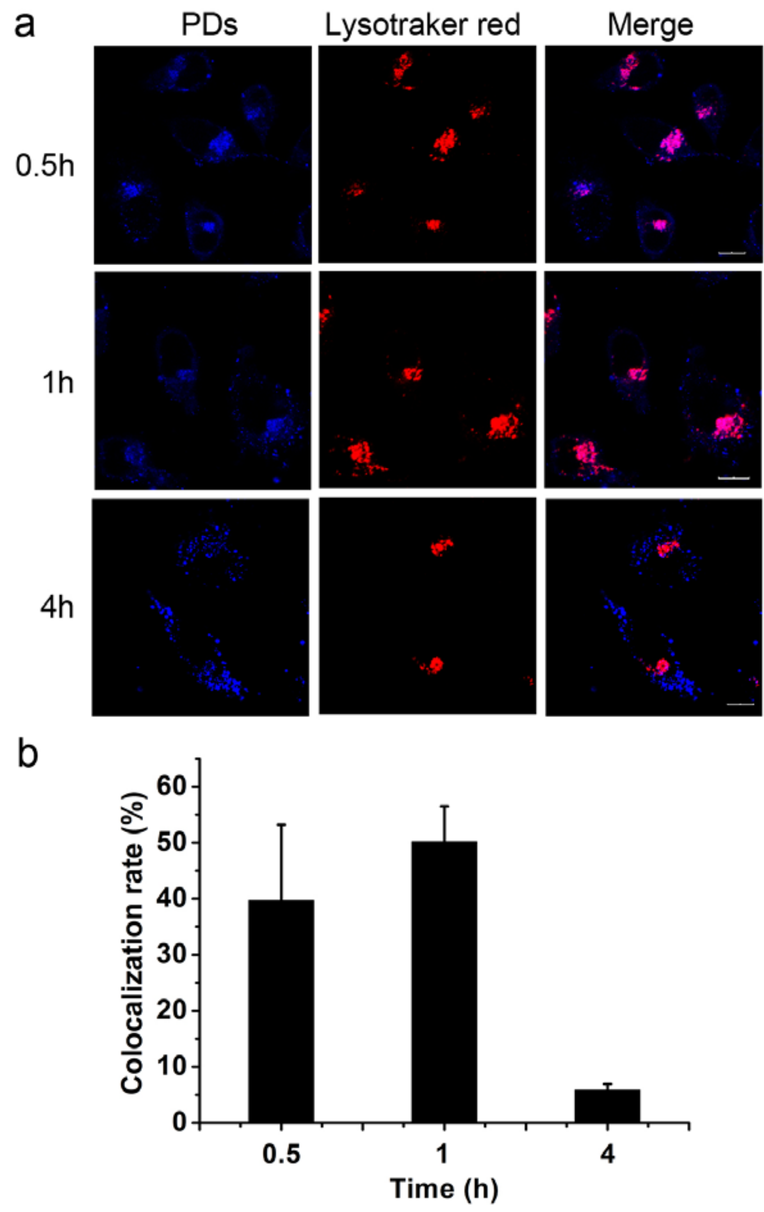


Fig. S7. (a) In vitro cellular imaging of MCF-7 cells incubated with PDs for various time points. Lysosomes were stained with lysotracker red (Molecular Probes, USA) for 30 min. Scale bar: 10 μm. (b) The colocalization rate between PDs and endosomes/lysosomes were quantified (n=15).

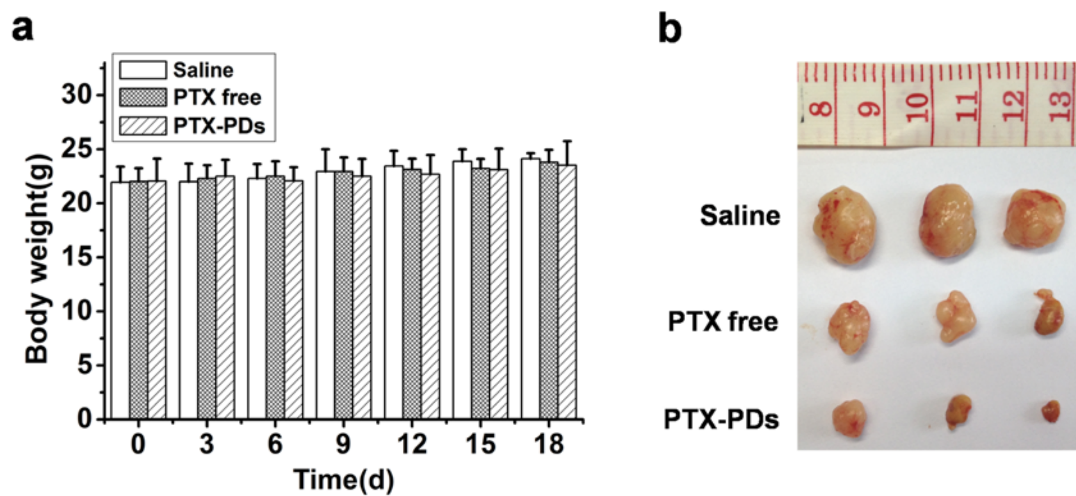


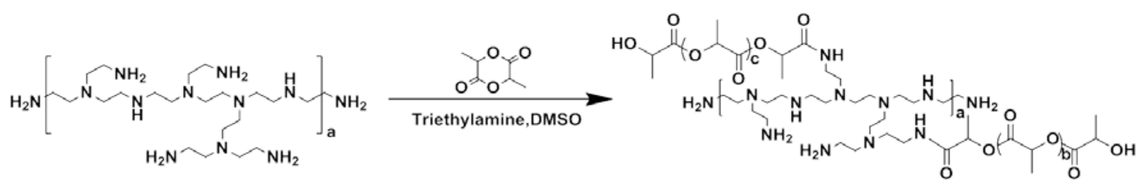
Fig. S8. Body weight of MCF-7 tumor-bearing female nude mice treated with saline, PTX or PTX-loaded PDs (a) and representative images of tumors from mice treated with saline, free PTX, or PTX-loaded PDs (b) . Data are presented as the mean ± standard deviation (n= 5).

Table S1. The effects of formulation parameters on drug-loading content and encapsulation efficiency (n=3)

Copolymer/PTX mass ratio		**10/1**	**20/1**	**50/1**	**100/1**
0.5% PVA	Loading Content(%)	3.97±0.21	2.51±0.37	0.64±0.15	0.45±0.17
	Encapsulation efficiency(%)	17.2±4.8	30.4±5.3	40.8±1.3	49.2±2.4
1% PVA	Loading Content(%)	5.04±0.10	2.41±0.08	0.79±0.11	0.50±0.17
	Encapsulation efficiency(%)	20.7±3.2	31.3±1.6	48.7±1.4	55.1±1.2
2% PVA	Loading Content(%)	5.11±0.26	3.27±0.18	0.83±0.22	0.58±0.12
	Encapsulation efficiency(%)	22.3±6.1	32.4±2.4	50.9±1.7	61.7±3.5

Table S2. Effects of dispersion solutions on particle size, poly-dispersity index (PDI)

Sample	Dispersion solutions	Size (nm)	PDI
Blank PDs	H_2O	217.5±2.7	0.140
	PBS	226.7±3.1	0.128
	DMEM+10%FBS	197.1±4.8	0.169
PTX-loaded PDs	H_2O	243.1±3.5	0.112
	PBS	247.6±4.4	0.149
	DMEM+10%FBS	205.6±5.7	0.166



Scheme S1. The synthetic route for PEI-PLA copolymer