# Theranostic nanoparticles enabling the release of phosphorylated gemcitabine for advanced pancreatic cancer therapy

## Supplementary information

1.1 Synthesis of pGEM

To the mixed $(MeO)_3PO$ (34 mL) and $POCl_3$ (23 mL), GEM (1.5 g) was added at 5 $^oC$ and stirred for 2 h. Then, aqua astricta (100 mL) was added to the above mixture under stirring to quench the reaction. $CHCl_3$ (100 mL×2) was used to extract the mixture, where the pH of aqueous layer was adjusted to 6.5 with $NH_4OH$ under 30 $^oC$. The aqueous layer was extracted with $CHCl_3$ (100 mL) once again. The aqueous phase was freezed-dried to give solid, which was re-dissolved into methanol (100 mL) and stirred for 1 h. The residual solid was filtered off and the solvent of the filtrate was removed under vacuum to give the crude product, which was submitted to a column (isopropyl alcohol:$NH_4OH$:$H_2O$=7:2:1, v:v:v) to obtain product containing organic salts. The solid was re-dissolved into ethanol and further eluted through ion exchange resin to give T. M. pGEM (800 mg) as a white solid. The pGEM was characterized by $^1H$ NMR (400 MHz) and $^{13}C$ NMR (Fig. S1 and S2).

1.2 Synthesis of the amphphilic ligand

Synthesis of Lys(*Z*)-NCA: Cbz-*L*-Lysine (0.5 g, 1.78 mmol) and triphosgene (0.212 g, 0.713 mmol) were dissolved into anhydrous THF (5 mL) under r. t. and Ar. The mixture was stirred under 50 $^oC$ for 4 h, cooled to r. t., and filtered. The filtrate was slowly added to hexane (30 mL) under vigorous stirring, where the appeared white solid was filered and washed with cold hexane to give Lys(*Z*)-NCA, which is characterized by $^1H$ NMR spectrum (Fig. S3).

Synthesis of PEG2000-p(Lsy(*Z*)): MAL-PEG2000-$NH_2$ (130 mg, 0.065mmol) and Lys(Z)-NCA (100mg, 0.326mmol) were dissolved into anhydrous DMF (5 mL) and stirred under 50 $^oC$ and Ar for 48 h. The whole mixture was cooled to r. t., dialyzed against water for 48 h, and freeze-dried to give PEG2000-p(Lsy(Z)). The Lsy(Z)'s polymerization degree was calculated to be n=10 based on the $^1H$ NMR spectrum (Fig. S4).

Synthesis of PEG2000-p(Lys(Z))-OA: PEG2000-p(Lys(*Z*))$_{10}$-$NH_2$ (88.7 mg), oleic acid (68 μL), 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 82 mg), 1-hydroxybenzotriazole (HOBT, 28.8 mg) and *N*,*N*-Diisopropylethylamine (DIPEA, 35 μL) were dissolved into anhydrous DMF (4 mL) under $N_2$ and stirred at r. t. for 24 h. Upon the completion of the reaction, the crude was seperated on a LH-20 Sephadex gel chromatography (methanol:$CH_2Cl_2$=1:2, v:v) to collect the band that has a UV absorbance at wavelength of 256 nm. The solvent was removed under vaccum and the residual solid was dialyzed against DMF for 24 h and water for 24 h. The obtained liquid was freezed-dried to give crude PEG-pLys(*Z*)-oleic acid, which was further purified by a precipitation method in cold ethyl ether (30 mL×3) to give pure PEG-pLys(*Z*)-oleic acid (93%) as a white wax. $^1$H NMR spectrum is used to characterize the product (Fig. S5).

Deprotection of PEG-pLys(*Z*)-oleic acid: PEG-pLys(*Z*)-oleic acid (100 mg) was dissolved into a mixed trifluoroacetic anhydride (10 mL) and 33% hydrobromic acid solution in acetic acid (0.5 mL), and stirred under r. t. for 3 h. The reaction mixture was directly dialyzed against water for 24 h to give PEG-pLys(*Z*)-oleic acid as a yellowish wax. $^1$H NMR spectrum of deproteced PEG-pLys(*Z*)-oleic acid is shown in Fig. S6.

1.3 Synthesis of $NaLuF_4$:Nd core nanoparticles

In a typical synthesis, 1 mmol $LnCl_3$ (Ln: Lu and Nd) with the molar ratio of 90:10 was added to a 100 mL three-necked flask. Then, 8 mL oleic acid and 15 mL 1-octadecene were supplemented. The mixture was heated to 90 °C with stirring for 20 min and then heated to 150 °C for 30 min with degassing to dissolve the $LnCl_3$ and form a transparent solution. After cooling down the solution to 50 °C, 5 ml methanol solution containing $NH_4F$ (4 mmol) and NaOH (2.5 mmol) was added dropwise into the system under stirring. After degassing for 20 min at 90 °C, the mixture was heated to 300 °C as soon as possible and kept at this temperature under argon for 1 h. When the mixture was cooled down to room temperature, nanoparticles were precipitated by adding 20 mL ethanol and cyclohexane (1:1) mixed solution and collected by centrifugation. After washing with ethanol and cyclohexane for three times, hexagonal phase $NaLuF_4$:Nd nanoparticles were finally collected and re-dispersed in 3 ml 1-octadecene.

1.4 Synthesis of $NaLuF_4$:Nd@$NaLuF_4$ core-shell nanoparticles

$NaLuF_4$:Nd@$NaLuF_4$ nanoparticles were prepared by epitaxial growth of $NaLuF_4$

layer on $NaLuF_4$:Nd through a similar procedure. 1.0 mmol $LuCl_3$ was added into a mixed solution of 8 mL oleic acid (OA) and 15 mL octadecene (ODE). The mixture was heated to 90 °C with magnetic stirring for 20 min. After that, the mixture was heated to 150 °C for 30 min to form transparent solution. After that, 3 mL 1-octadecene solution containing the as-prepared $NaLuF_4$:Nd nanocrystals was mixed with the solution. The following procedures were the same as the synthesis of core nanoparticles. The as-obtained $NaLuF_4$:Nd@$NaLuF_4$ nanoparticles were washed with ethanol and cyclohexane for three times and then dispersed in 10 ml cyclohexane for further use.

1.5 Synthesis of $NaLuF_4$:Yb,Er@$NaLuF_4$ core-shell nanoparticles

$NaLuF_4$:Yb,Er@$NaLuF_4$ core-shell nanoparticles were used to prepare VPNS for cell imaging. The synthesis of $NaLuF_4$:Yb,Er@$NaLuF_4$ core-shell nanoparticles were similar to the method for making $NaLuF_4$:Nd@$NaLuF_4$ core-shell nanoparticles except that the lanthanide elements used for making core nanoparticles were Lu, Yb and Er of which the molar ratio were 78:20:2.

2 Calculation of photothermal efficiency

The total energy conservation for the system can be expressed by Eq. 1.

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_{cs} + Q_B - Q_{sur} \quad (1)$$

where $m$ and $C_p$ are the mass and heat capacity of water respectively, $T$ is the solution temperature, $Q_{cs}$ is the heat generated from nanoparticles, $Q_B$ is the baseline energy induced by the cuvette, and $Q_{sur}$ is the heat conduction by air.

$Q_{cs}$ is induced by PdPc under irradiation of 730 nm laser:

$$Q_{cs} = I\left(1 - 10^{-A_{730}}\right)\eta \quad (2)$$

where $I$ is the laser power, $\eta$ is the conversion efficiency from incident laser energy to thermal energy, and $A_{730}$ is the absorbance of nanoparticles at wavelength of 730 nm. $Q_B$, indicating the heat dissipated from light absorbed by the cuvette, was measured to be 26.3 mW by using pure water without VPNS. Moreover, $Q_{sur}$ is in proportion to temperature for the outgoing thermal energy, which is given by Eq. 3:

$$Q_{sur} = hS(T - T_{amb}) \quad (3)$$

where $h$ is the heat transfer coefficient, $S$ is the surface area of the container, and $T_{amb}$ is the ambient temperature.

According to Eq. 3, when the system temperature reaches a maximum, the heat input is equal to heat output:

$$Q_{CS} + Q_B = hS(T_{max} - T_{amb}) \quad (4)$$

where $T_{max}$ is the equilibrium temperature. The 730 nm laser heat conversion efficiency ($\eta$) can be determined by substituting Eq.2 for $Q_{CS}$ into Eq. 4 and rearranging to get Eq. 5:

$$\eta = \frac{hS(T_{max} - T_{amb}) - Q_B}{I\left(1 - 10^{-A_{730}}\right)} \quad (5)$$

where $Q_B$ was 26.3 mW, and the ($T_{max}$-$T_{amb}$) was 12.6 °C according to Figure S7, $I$ is 300 mW/cm$^2$, $A_{730}$ is the absorbance (0.65) of nanoparticles at 730 nm. $hS$ is calculated by introducing $\theta$, is defined as the expression below:

$$\theta = \frac{T - T_{amb}}{T_{max} - T_{amb}} \quad (6)$$

and a sample system time constant $\tau_s$

$$\tau_s = \frac{\sum_i m_i C_{p,i}}{hs} \quad (7)$$

which is substituted into Eq. 4 and rearranged to yield

$$\frac{\mathrm{d}\theta}{\mathrm{d}t} = \frac{1}{\tau_s}\left[\frac{Q_{cs} + Q_B}{hS(T_{max} - T_{amb})} - \theta\right] \quad (8)$$

At the cooling stage of the aqueous dispersion of the nanoparticles, the light source was shut off, the $Q_{CS}$ +$Q_B$ = 0, reducing the Eq. 9

$$\mathrm{d}t = -\tau_s \frac{\mathrm{d}\theta}{\theta} \quad (9)$$

and integrating, giving the expression

$$t = -\tau_s \ln\theta \quad (10)$$

Time constant, $\tau_s$, for heat transfer from the system is determined to be 365.1 s by applying the linear time data from the cooling period *vs* negative natural logarithm of $\theta$ (Figure S7). In addition, the $m$ is 1 g and the $C$ is 4.2 J/g. According to Eq. 7, the $hS$ is deduced to be 11.5 mW/°C. Substituting $hS$ = 11.5 mW/°C into Eq. 5, the 730 nm

laser heat conversion efficiency ($\eta$) of nanoparticles is 51.0 %.

3 Cellular uptake observed by fluorescent confocal imaging

MIA PaCa-2 cells were cultured in DMEM culture medium containing 10% FBS, then trypsin was used to digest the cells to obtain a suspension with a $1\times10^5$ $mL^{-1}$ density, 2 mL of which was transferred to a dish (35 mm diameter) containing with a slide (14 mm diameter) and cultured at 37 $^oC$ overnight. PBS was used to wash the adherent cells and nanoparticle solution (2 mL, 200 μg/mL) was added to the dish and cultured at 37 $^oC$ for 4 h. The medium was removed and the slide was rinsed with PBS thrice. The cells were observed under up-converted fluorescent confocal imaging system with an 808 nm/19 mW excitation light source. The objective lens was ×40 times and the wavelength of the fluorescence collector was ranged from 510-560 nm.

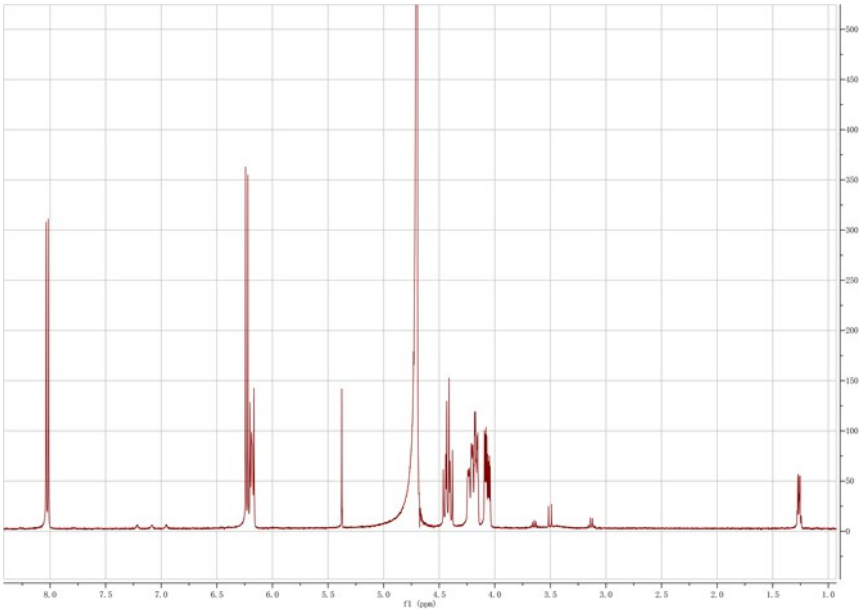

Figure S1. 1H-NMR spectrum of GEM-MP.

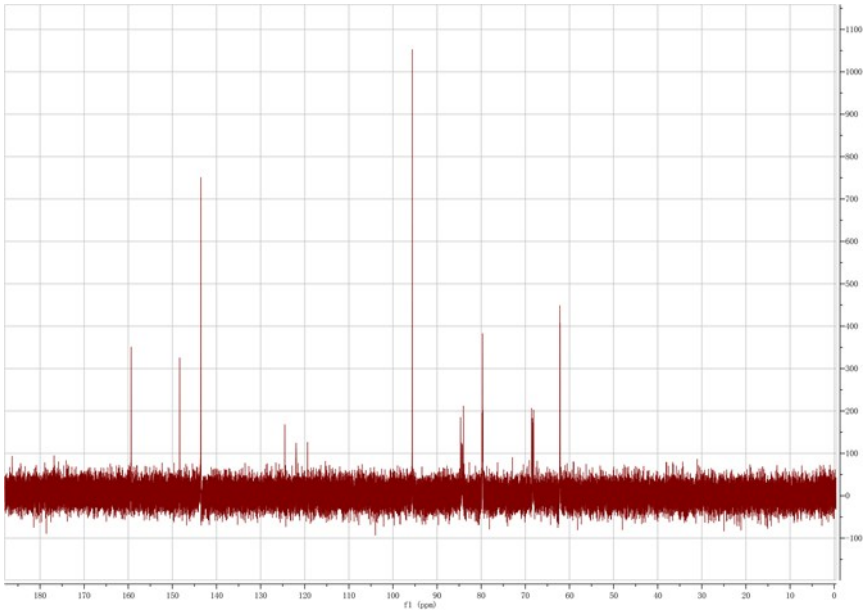

Figure S2. Spectrum of carbon source for GEM-MP.

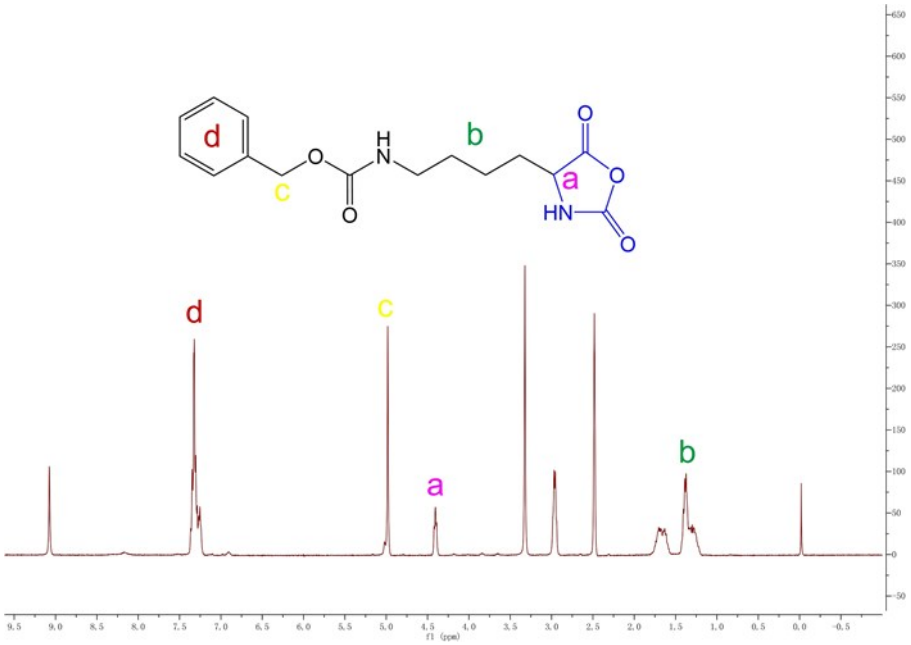


Figure S3. 1H-NMR spectrum of Lys(*Z*)-NCA.

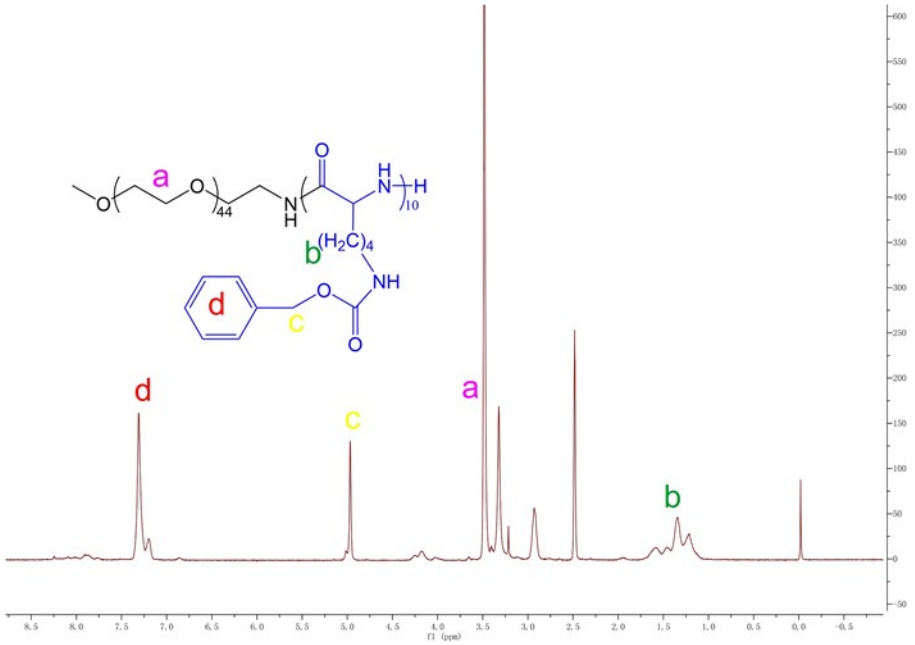


Figure S4. 1H-NMR spectrum of PEG2000-pLys(*Z*).

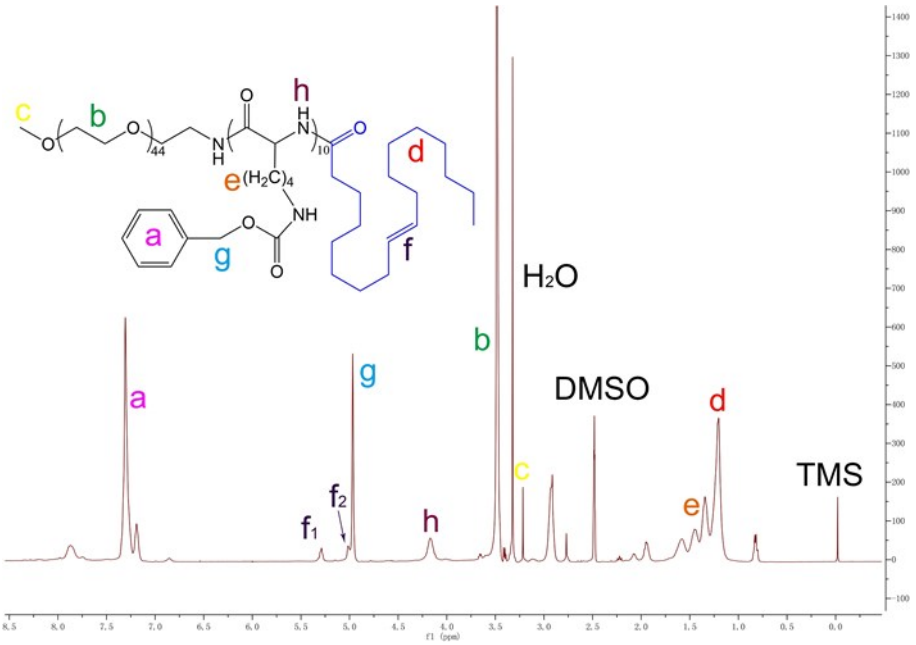


Figure S5. 1H-NMR spectrum of protected PEG2000-pLys(*Z*)-OA

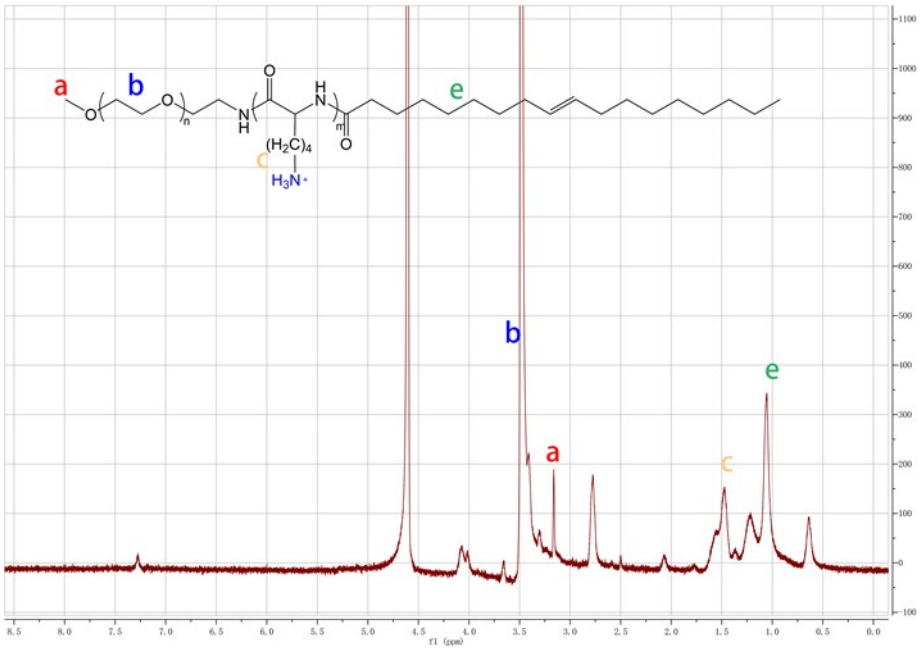


Figure S6. 1H-NMR spectrum of deprotected PEG2000-pLys(*Z*)-OA

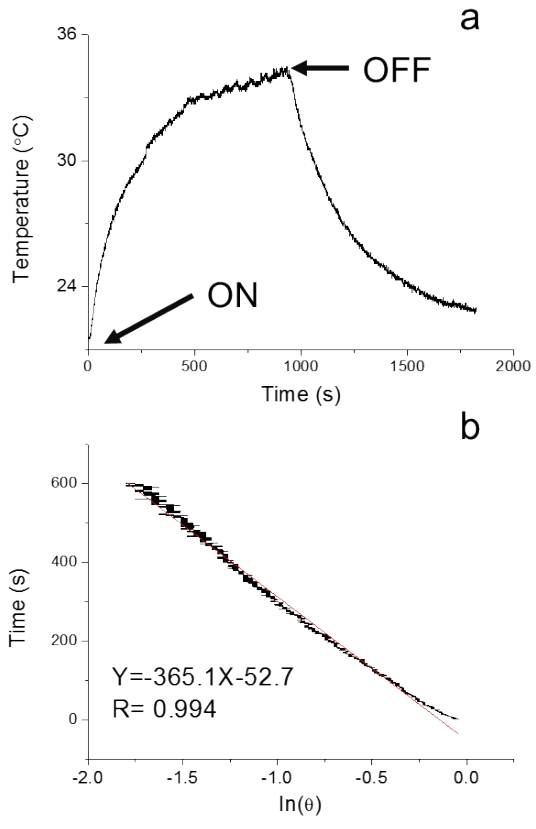


Figure S7. (a) Curve of photothermal temperature of VPNS in aqueous solution. The power density of the 730 nm laser is 0.3 W $cm^{-2}$, and the laser is turned off (OFF) after 1000 s irradiation to allow the solution to cool. (b) The time-dependent curve of ln (θ) in the natural cooling section in aqueous solution, and the heat transfer time constant $\tau_s$ can be obtained by linear fitting.

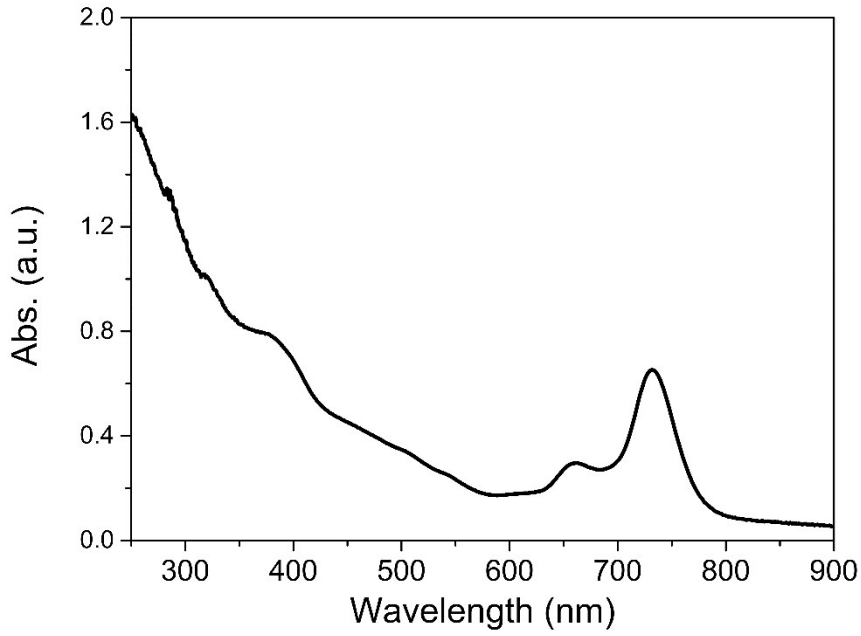


Figure S8. The UV absorption spectra showed that Pd phthalocyanine had an absorption peak at 730 nm.

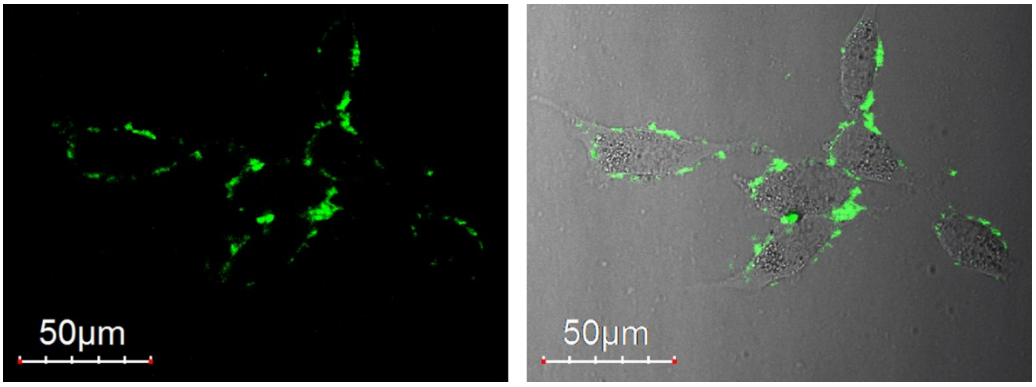


Figure S9. The confocal microscopy showed nanoparticles were distributed in the cells equably after incubated with the cells.

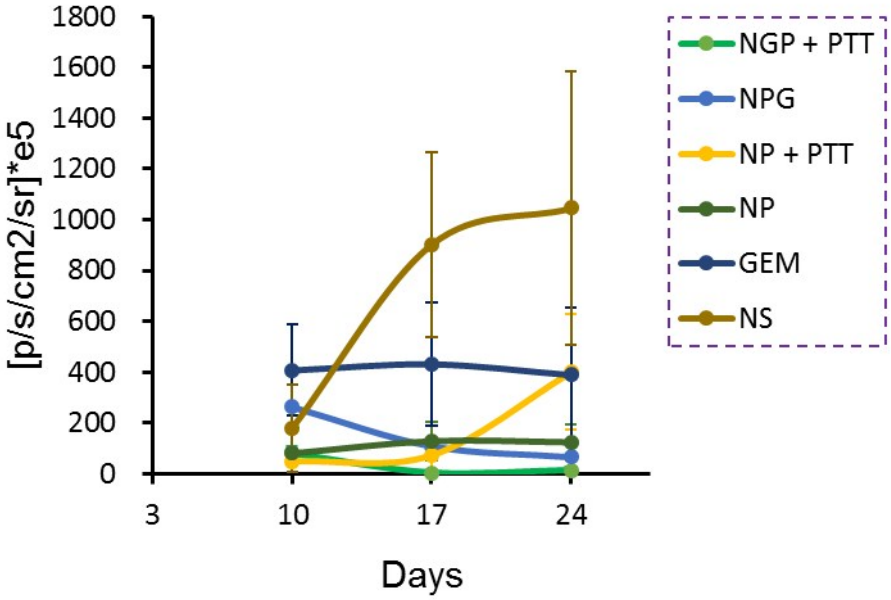


Figure S10. Quantification of signals from the entire abdominal region of each mouse. Data are represented as means ± SD. (n = 4).