Supporting materials for

Morphological Insights in Oxidative Sensitive Nanocarriers Pharmacokinetics, Targeting, and Photodynamic Therapy

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1. Main Experimental methods

1.1. Stability

To study the stability of Ce6@NPs, we incubated them in water, PBS and cell culture medium. Alteration of size and zeta potential were detected *via* DLS (Omni, Brookhaven Instrument, USA). Briefly, Ce6@NPs were incubated in above three conditions for 2 hours, 6 hours, 24 hours, 48 hours and 72 hours. At each time point, samples were measured *via* DLS.

1.2 Cell culture

HepG2 (human liver carcinoma cell line) and QSG (human hepatic cell line) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). And they were cultured in the cell incubator under a humidified atmosphere at 37 ℃ (5% CO_2, 95% air). The DMEM medium was used to culture QSG and HepG2 cells (containing 1% (v/v) penicillin-streptomycin, and 10% (v/v) fetal bovine serum). The cells out of the liquid nitrogen tank were passaged at least twice for experimental use.

1.3 Cytotoxicity assessment

HepG2 and QSG cells were seeded in 96-well plates and cultured in a normal oxygen incubator (5% CO_2, 37 °C). After 24 hours, PEG5k-$PMET_{5/40/120}$ at an equivalent concentration of 0–200 μg/mL was added in each well (n = 5 for each group). After 24 hours of incubation with contrast agents, the CCK-8 (Med Chem Express, USA) assay was performed to measure the cytotoxicity of experimental and control groups.

1.4 Endocytosis pathways

To clarify the endocytic pathway of NPs, HepG2 cells were seeded into 12-well plates. Next, cells and inhibitors (10 μg/mL chlorpromazine, 10 μM 2-deoxy-d-glucose, 10 μM colchicine, and 50 mM NH_4Cl) were incubated for 30 minutes at 37 °C. After removing the above inhibitors, Ce6@NPs at a Ce6 concentration of 1 μg/mL were added to the pre-incubated cells and incubated for an additional 3 hours. Another group of cells was pre-incubated for 30 minutes at 4 °C, followed by the addition of Ce6@NPs for an additional 3 hours. Cells cultured at room temperature without any treatment were used as controls. After completing the above treatment steps, the cells were collected, washed twice with PBS, and the fluorescence intensity inside the cells was detected by flow cytometry.

1.5 Sub-organelle colocalization experiment

HepG2 cells were inoculated on a glass substrate and incubated for 1 day. Micelle, worm and vesicle

were incubated with the cells. The concentration was determined based on the Ce6 (10 μg/mL) content. After incubation for 3 hours, organelle staining reagents were added, including ER-Tracker Green (Beyotime, C1042S), Lyso-Tracker Green (Beyotime, C1047S), Mito-Tracker Green (Beyotime, C1048), and Golgi-Tracker Green (Beyotime, C1045S). After staining, the cells were washed twice, followed by Hoechst 33342 staining (Yeasen, China) for 10 minutes, and then washed twice with PBS. Confocal microscopy (CLSM, Nikon, Japan) was used to acquire images.

1.6 Physiological based pharmacokinetics (PBPK) model's parameter fitting and ranking

The PBPK model includes numerous parameters. To explore the parameter space and fit the parameter, we utilized particle swarm optimization (PSO) implemented via the Python package PySwarms, genetic algorithm (GA) via the Python package DEAP, and simulated annealing (SA) via the Python package SciPy. After we find out the parameter for different particle type, we analysised the parameter sensitivity. Sensitivity analysis can be divided into two categories: local sensitivity analysis and global sensitivity analysis[1]. The sensitivity can be calculated as follows:

$$SC = \frac{(AUC_{0-\infty}^{'} - AUC_{0-\infty})/AUC_{0-\infty}}{(Par^{'} - Par)/Par}.$$

where SC is the sensitivity; $AUC_{0-\infty}$ and $AUC_{0-\infty}^{'}$ is the area under curve before and under perturbed, respectively; and $Par^{'}$ and $Par$is the perturbed value and reference value of the parameter under investigation.

2. Supporting Figures

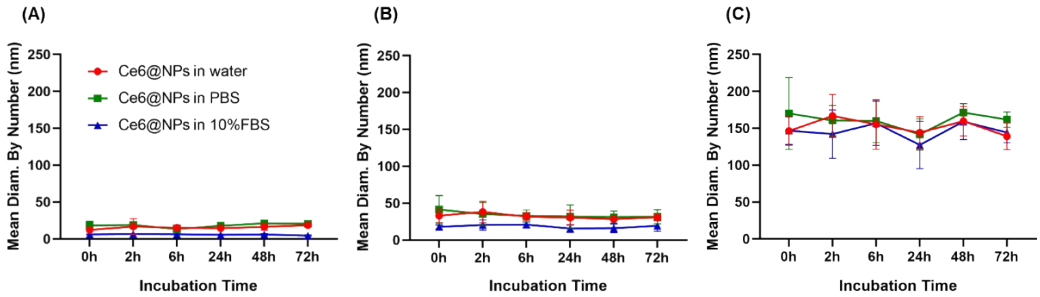


Fig. S1 (A-C) The average size of Ce6@PEG5k-PMET$_{5/40/120}$ with time in water, PBS and 10% FBS.

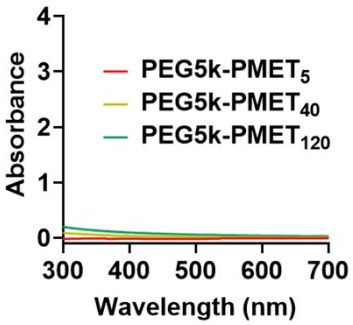


Fig. S2 Absorption spectra of PEG5k-PMET$_{5/40/120}$.

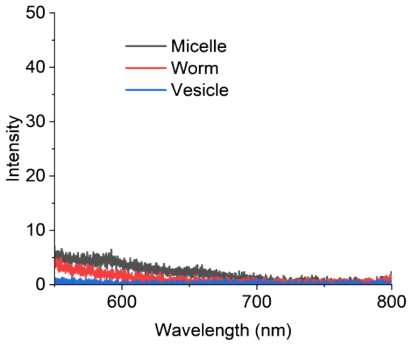
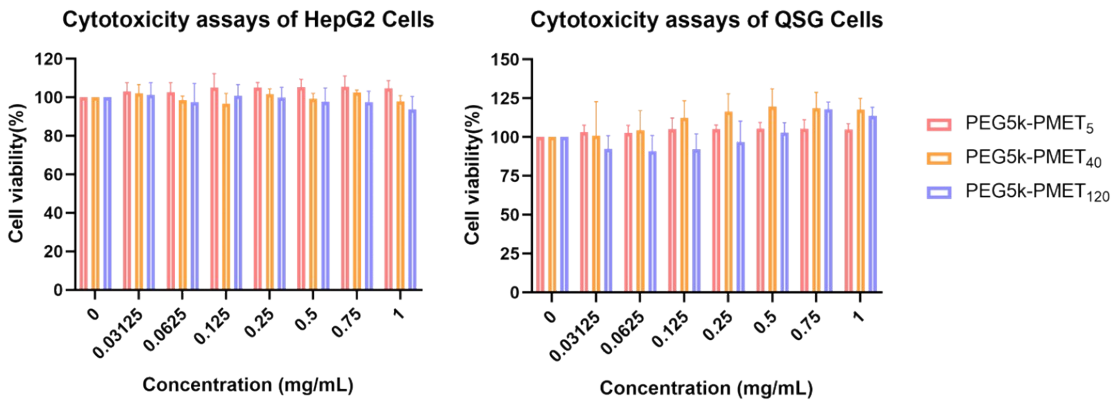


Fig. S3 Fluorescence spectra of PEG5k-PMET$_{5/40/120}$.



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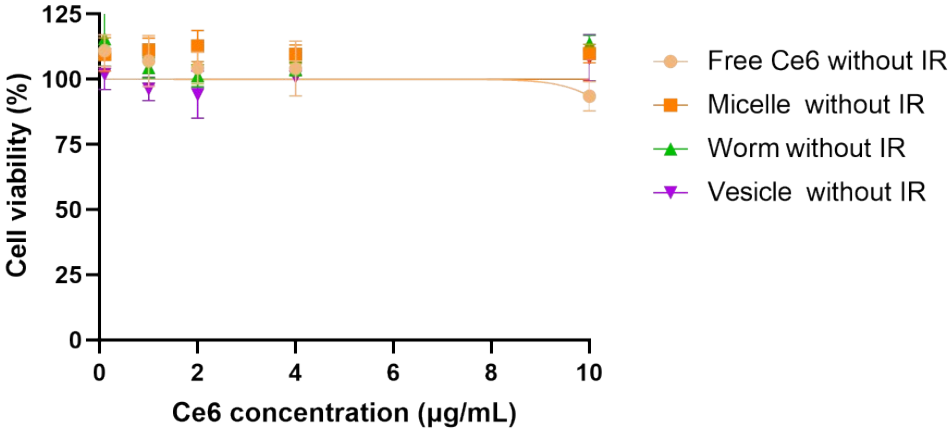
129 **Fig. S4 *In vitro* cytotoxicity of PEG5k-PMET$_{5/40/120}$ in QSG and HepG2 cells.**



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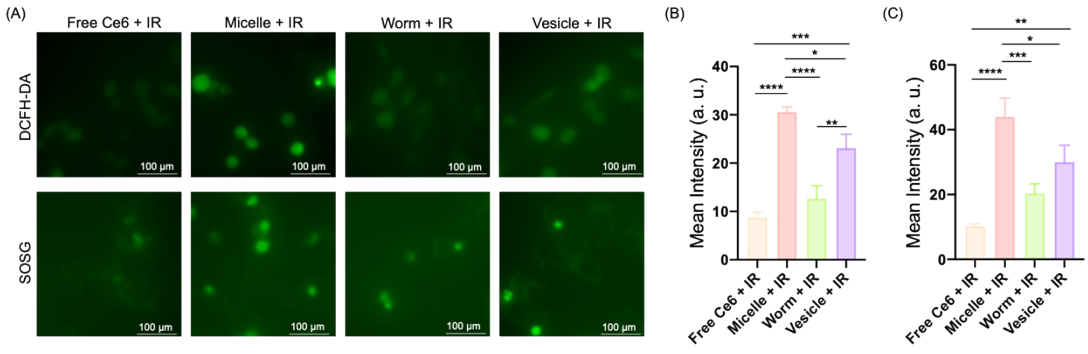
131 **Fig. S5 *In vitro* cytotoxicity of Ce6@NPs in HepG2 cells without laser irradiation.**



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133 **Fig. S6 (A) Fluorescence images of HepG2 cells stained with DCFH-DA and SOSG after treatment with**

134 **Ce6@NPs for 24 hours. Quantification of the fluorescence intensity of DCFH-DA (B) and SOSG (C) in**

135 **HepG2 cells treated with Ce6@NPs for 24 hours.**

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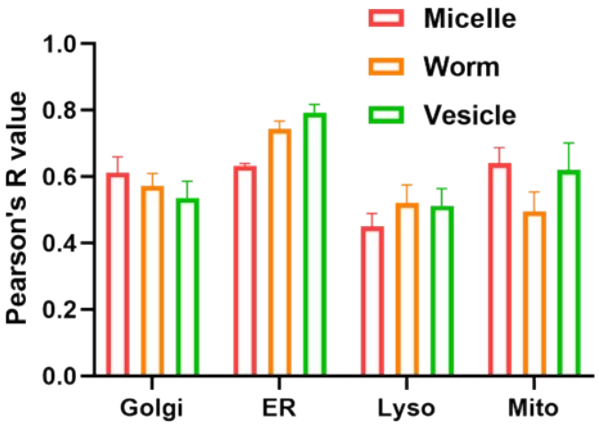


Fig.S7 Histogram results of Pearson coefficients of micelles, worms, and vesicles with Golgi (Golgi bodies), ER (endoplasmic reticulum), Lyso (lysosomes), and Mito (mitochondria) in HepG2 cells.

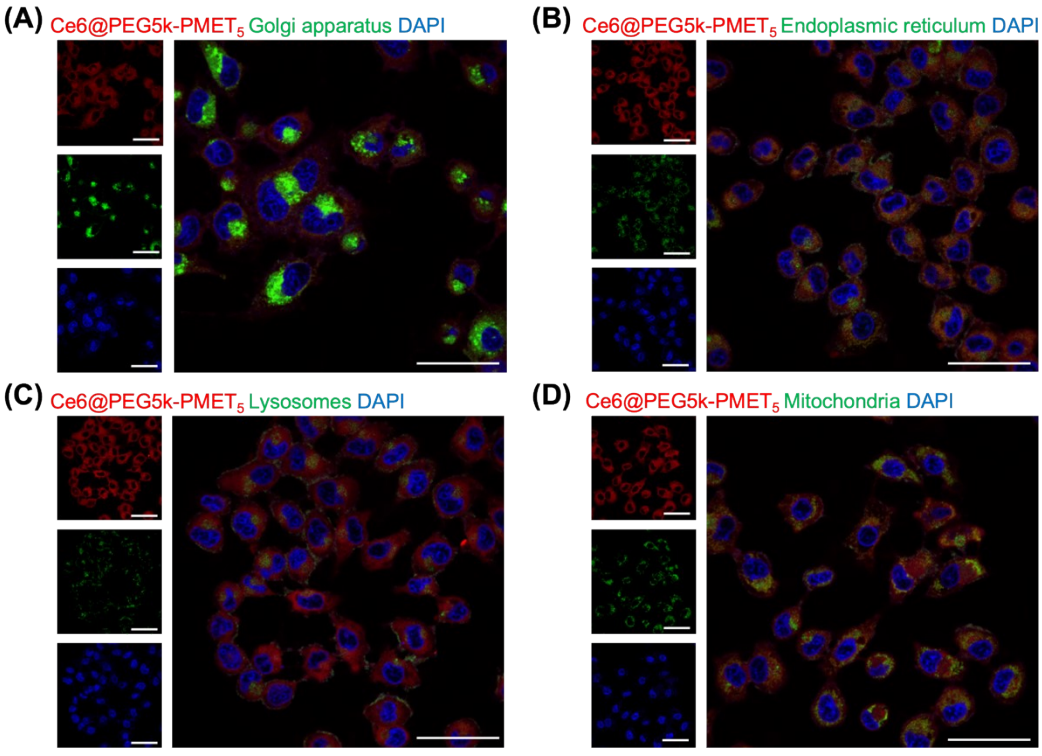


Fig.S8 The colocalization of micelle and organelles. Scale bar = 50 μm. (A) Golgi apparatus; (B) Endoplasmic reticulum; (C) Lysosomes; (D) Mitochondria.

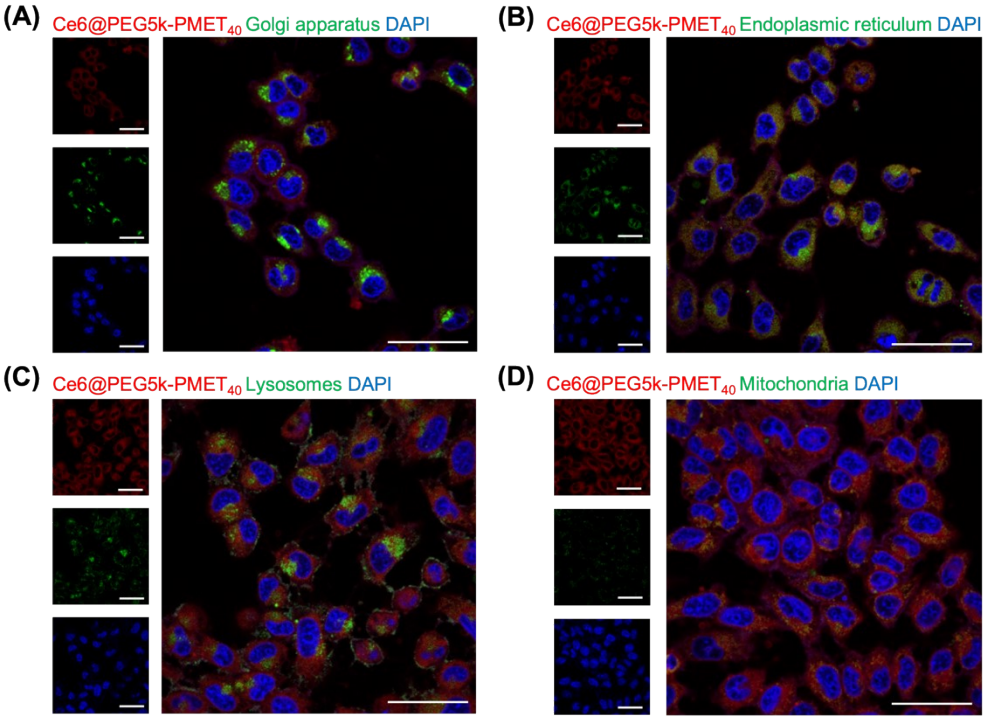


Fig.S9 The colocalization of worm and organelles. Scale bar = 50 μm. (A) Golgi apparatus; (B) Endoplasmic reticulum; (C) Lysosomes; (D) Mitochondria.

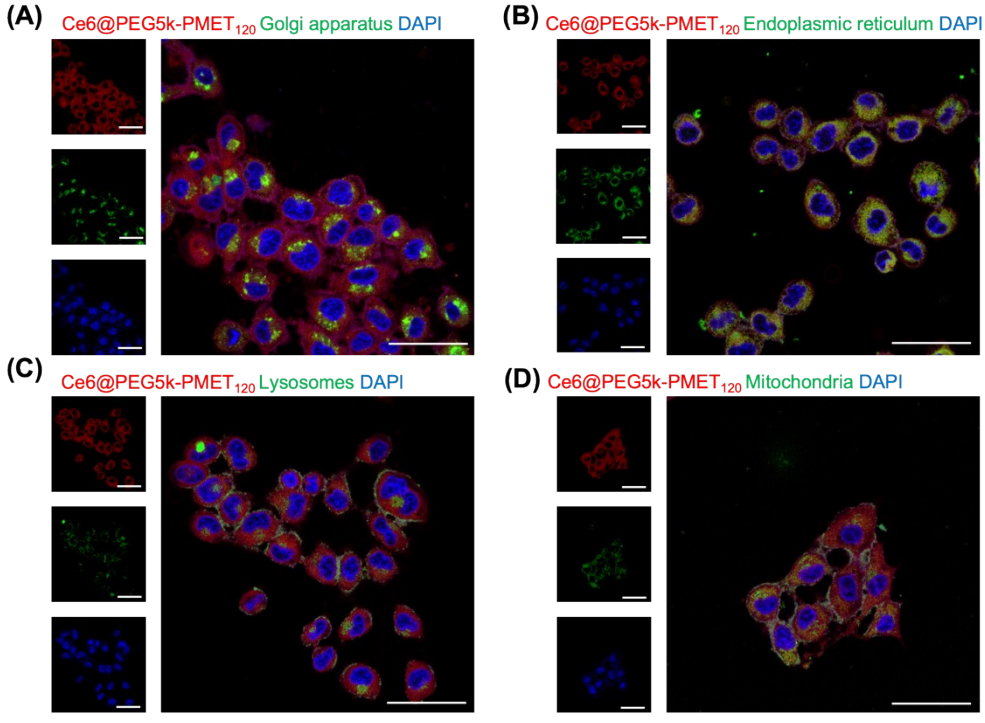
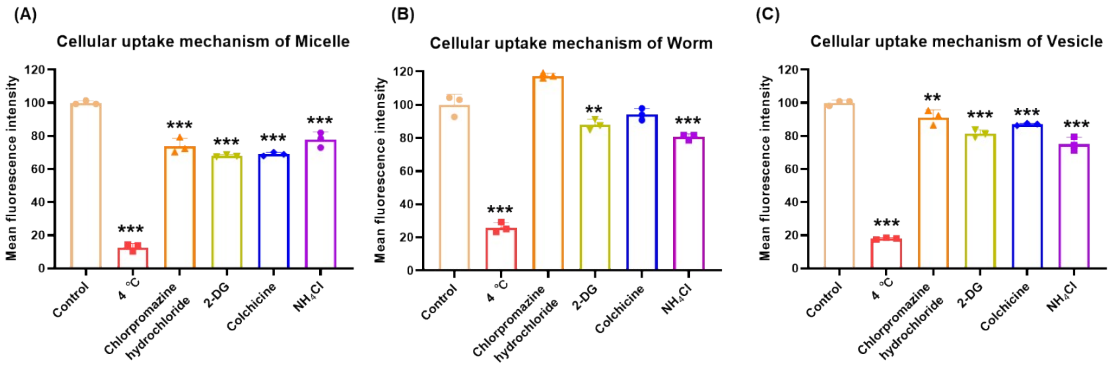


Fig.S10 The colocalization of vesicle and organelles. Scale bar = 50 μm. (A) Golgi apparatus; (B) Endoplasmic reticulum; (C) Lysosomes; (D) Mitochondria.



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154 **Fig.S11 Flow cytometry detection of Effects of different endocytic inhibitors and temperature (4 ℃)**

155 **on the cellular uptake of micelle(A), worm(B), and vesicle(C).**

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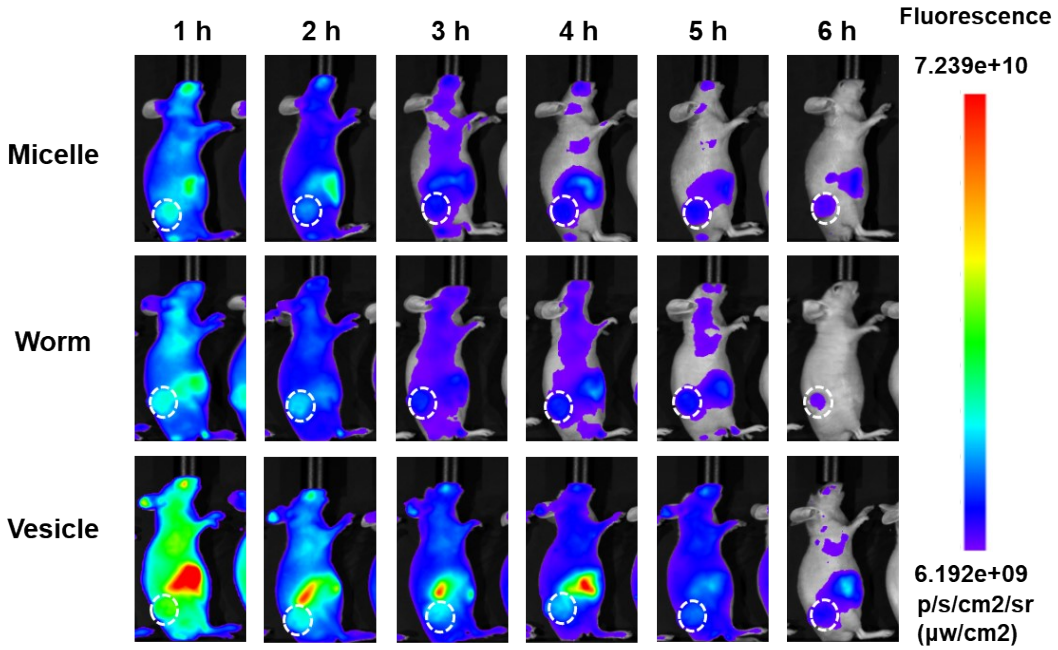

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158 **Fig.S12 *In vivo* fluorescent images of nude mice bearing HepG2 tumors treated with NPs@Ce6 (the**

159 **concentration of Ce6 at 0.5 mg/mL) at different time-points.**

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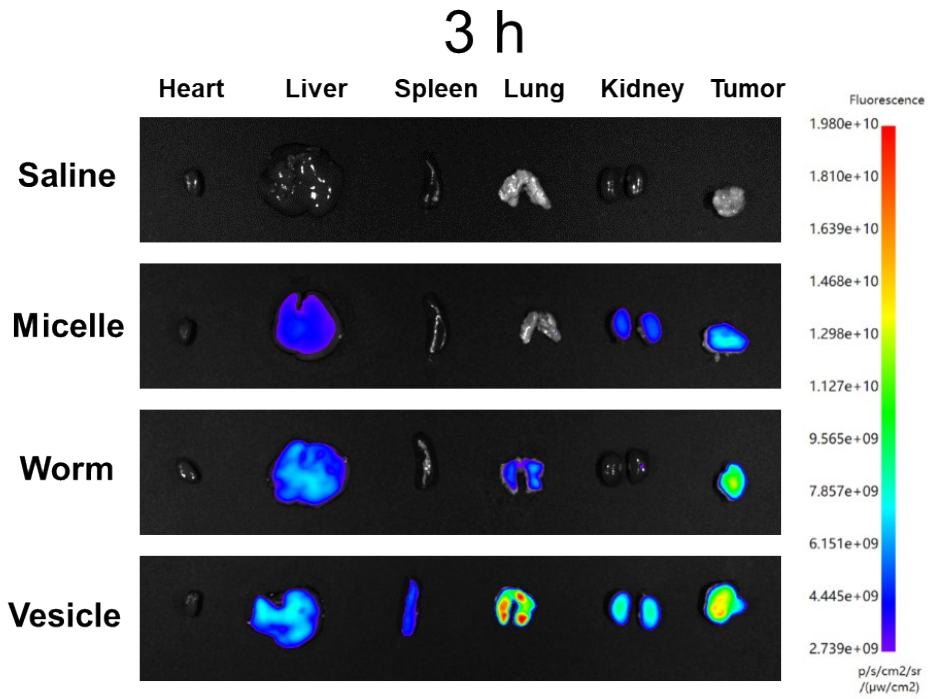

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162 **Fig.S13 *Ex vivo* fluorescent images for harvested tumors and major organs at the 3 hours.**

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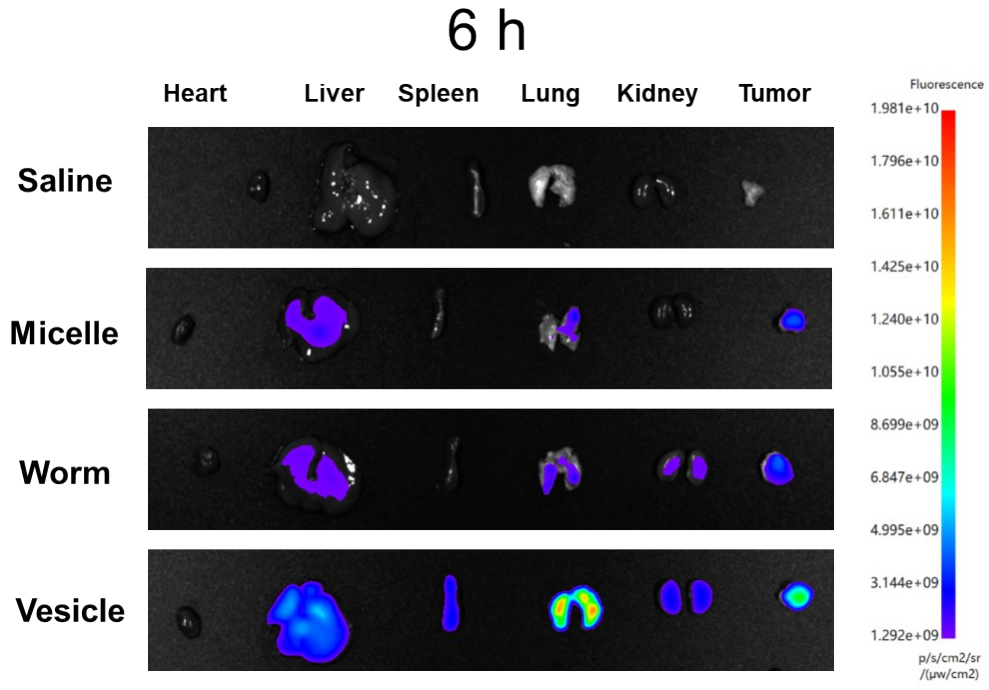

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165 **Fig.S14 *Ex vivo* fluorescent images for harvested tumors and major organs at the 6 hours.**

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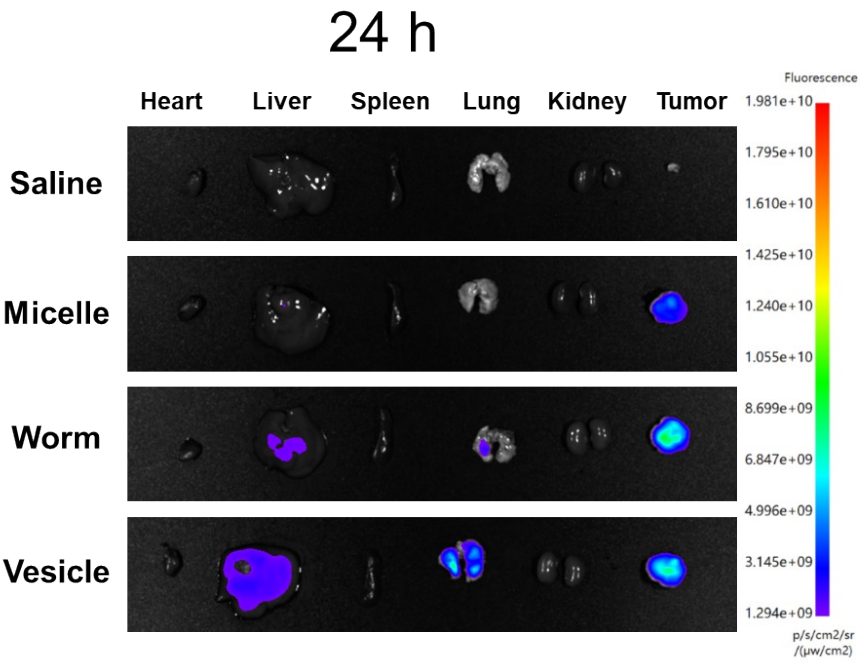

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168 **Fig.S15 *Ex vivo* fluorescent images for harvested tumors and major organs at the 24 hours.**

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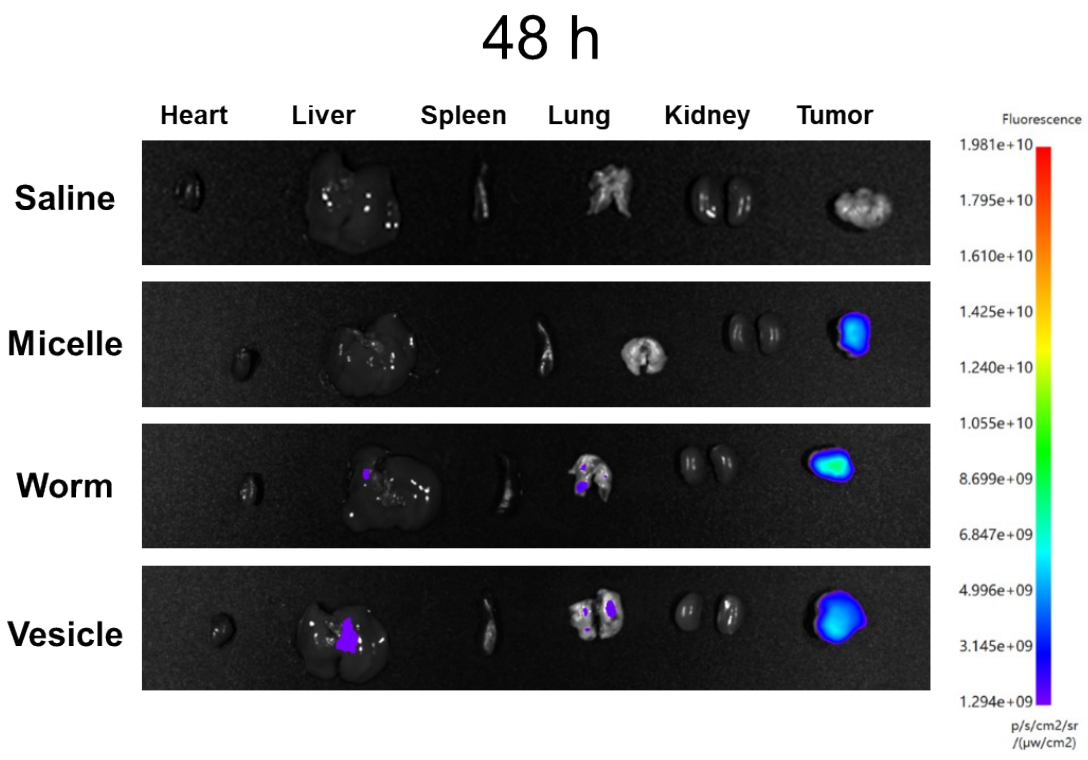

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171 **Fig.S16 *Ex vivo* fluorescent images for harvested tumors and major organs at the 48 hours.**

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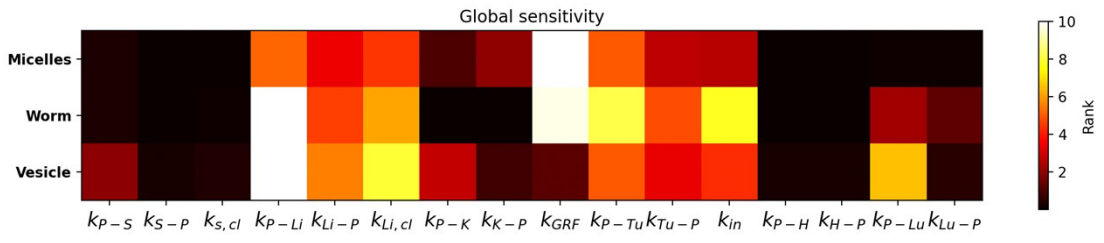

Fig. S17. Parameter rank based on global sensitivity in the PBPK model. The parameter with the highest sensitivity in the model is ranked 10, while the one with the lowest sensitivity is ranked 0.

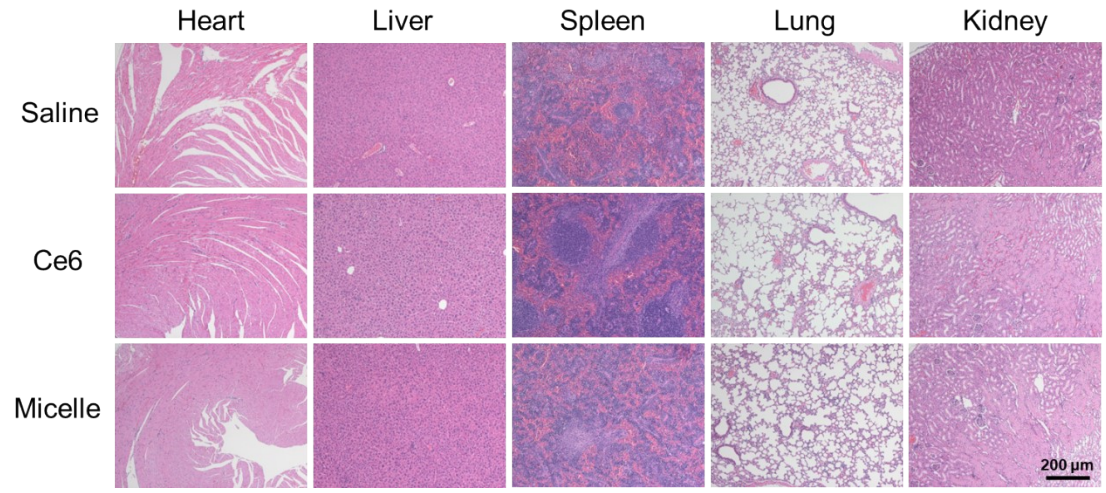

Fig.S18 H&E staining of the heart, liver, spleen, lung, and kidney after various treatments.

3. Supporting Tables

Table S1. Pharmacokinetic parameters for mice treated with NPs@Ce6 and free Ce6 by fitting the data to a non-compartment model by PKSolver 2.0 software.

Parameter	$T_{1/2}$ (min)	$AUC_{0-\infty}$ (μg/mL·min)	CL (L/min/kg)	$MRT_{0-\infty}$ (min)
Micelle	313	794	0.126	378
Worm	563	898	0.111	518
Vesicle	428	729	0.137	459

1. P. Dogra, J. D. Butner, J. Ruiz Ramirez, Y. L. Chuang, A. Noureddine, C. Jeffrey Brinker, V. Cristini and Z. Wang, *Comput Struct Biotechnol J*, 2020, **18**, 518-531.