Supporting Information

Hydrogen Peroxide-Activatable Nanoparticles for Luminescence Imaging and In-Situ Triggerable Photodynamic Therapy of Cancer

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Figure S1. Characterization of the CLP conjugate. (A-B) FT-IR (A) and $^1$H NMR (B) spectra of different materials. (C-D) MALDI-TOF mass spectra of PEG (C) and CLP conjugate (D).

In the FT-IR spectrum of the CLP conjugate, a new absorption peak related to carbonyl stretching appeared at 1739 cm$^{-1}$, indicating successful coupling reactions of Ce6, luminol, and PEG. In addition, the characteristic proton signals of PEG, luminol, and Ce6 were observed in the $^1$H NMR spectrum of CLP conjugate in DMSO-$d_6$. The MALDI-TOF spectrum of CLP conjugate in (D) demonstrated that most of the conjugate molecules showed $M_w$ of about 2.4 kDa, which is almost identical to the theoretical value.
Figure S2. Spectroscopy characterization of the CLP conjugate. (A-B) 3D fluorescence spectra of CLP conjugate (A) and Ce6 (B) in DMSO. (C-D) Fluorescence excitation (C) and fluorescence emission (D) spectra of CLP conjugate and Ce6 in DMSO. For excitation spectra, the emission wavelength was 670 nm, while the excitation wavelength was 401 nm for emission spectroscopy. (E) UV-visible absorption spectrum of CLP conjugate and Ce6 in DMSO.
Figure S3. $^1$H NMR spectroscopy characterization of CLP conjugate in D$_2$O or DMSO-D$_6$. 
Figure S4. Spectroscopy characterization of CLP nanoparticles in aqueous solutions. (A) 3D fluorescence spectrum of 0.02 mg/mL CLP nanoparticles in aqueous solution. (B-C) Fluorescence excitation (B) and fluorescence emission (C) spectra of 0.02 mg/mL CLP nanoparticles in aqueous solution. (D) UV-visible absorption spectrum of 0.02 mg/mL CLP nanoparticles in aqueous solution. (E-H) The effects of different concentrations of H$_2$O$_2$ (E), NAC (F), GSH (G), and CAT (H) on UV-visible spectra of 0.02 mg/mL CLP nanoparticles in aqueous solutions after 12 h of incubation. (I-L) The effects of different concentrations of NAC (I), GSH (J), CAT (K), and H$_2$O$_2$ (L) on fluorescence emission spectra of 0.02 mg/mL CLP nanoparticles in aqueous solutions after 12 h of incubation. For excitation spectra, the emission wavelength was 670 nm, while the excitation wavelength was 401 nm for emission spectroscopy.
**Figure S5.** Quantitative data showing the effects of H$_2$O$_2$ concentrations on luminescence of CLP nanoparticles at 0.5 mg/mL (0.108 mM) or luminol at the same dose of the luminol unit. Data are presented as the mean ± SEM (n = 3).
**Figure S6.** The luminescence spectrum of luminol and the absorption spectrum of Ce6. The luminescence spectrum of luminol was acquired in PBS, while the absorption spectrum of Ce6 was obtained in DMSO.
Figure S7. Changes in physicochemical properties of CLP nanoparticles in the presence of \( \text{H}_2\text{O}_2 \). (A-C) Changes in particle sizes (A), light scattering intensities (B), and \( \xi \)-potential values (C) at predefined time points after CLP nanoparticles were incubated with various concentrations of \( \text{H}_2\text{O}_2 \). (D) A typical TEM image showing nanoparticles obtained after incubation of CLP nanoparticles with 100 mM \( \text{H}_2\text{O}_2 \) for 24 h. Data are presented as the mean ± SEM (\( n = 3 \)).
Figure S8. Cellular uptake of CLP nanoparticles in 4T1 cells. (A-B) Representative flow cytometric profiles (left) and quantitative data (right) indicating time-dependent (A) or dose-response (B) cellular internalization of CLP nanoparticles by 4T1 cells. The dose of CLP nanoparticles was 21.6 μM to examine the time effect, while the dose-response effect was tested after 8 h of incubation. Data are presented as the mean ± SEM (n = 3).
Figure S9. The effect of different ROS scavengers on luminescence of CLP nanoparticles in 4T1 cells. (A-B) Representative luminescent images (left) and quantitative analysis (right) of luminescent signals of 4T1 cells treated with 10.8 μM CLP nanoparticles and in the presence of a superoxide anion scavenger Tempol (A) or a hydroxyl radical scavenger sodium formate (SF) (B). Data are presented as the mean ± SEM (n = 3).
Figure S10. Luminescence intensities of CLP nanoparticles as a function of the H$_2$O$_2$ concentration. This calibration curve was used to calculated the absolute quantity and concentration of H$_2$O$_2$ in each 4T1 cell.
Figure S11. Comparison of $^1\text{O}_2$ generation capacity of different materials in the presence of H$_2$O$_2$. Different formulations were incubated with 100 $\mu$M H$_2$O$_2$ in deuterium oxide, the relative $^1\text{O}_2$ generation capacity was determined by using a fluorescent probe SOSG. The concentration of CLP nanoparticles was 100 $\mu$g/mL, while the concentration of Ce6-PEG was 56 $\mu$g/mL that contained the same amount of Ce6 as CLP nanoparticles. In the luminol group, 3 $\mu$g/mL luminol sodium salt was used which contained the same amount of the luminol unit as CLP nanoparticles. Of note, CLP, Ce6-PEG, and luminol sodium salt were also dissolved in deuterium oxide. Data are presented as the mean ± SEM (n = 3). ***P < 0.001.
Figure S12. Quantification of intracellular ROS levels in three cancer cells after different treatments. (A-C) Representative flow cytometric profiles (left) and quantitative data (right) illustrating the relative intracellular ROS levels in 4T1 (A), HCT116 (B), and A549 (C) cells. In all cases, cells were treated with fresh medium in the control group, while the CLP group was incubated with CLP nanoparticles at 200 μg/mL (43.2 μM) for 4 h. Data are presented as the mean ± SEM (n = 3).
Figure S13. *In vivo* tumor therapy after intratumoral administration of CLP nanoparticles in mice bearing 4T1 tumors. (A) The treatment regimen for tumor therapy by intratumoral injection of CLP nanoparticles. (B) Digital photos of mice during 15 days of treatment. After establishment of 4T1 tumor-bearing mice by subcutaneous inoculation of 4T1 cells, CLP nanoparticles were administered by intratumoral injection at 3.25 mg/kg (CLP-L) or 6.5 mg/kg (CLP-H) Ce6 every 3 days.
Figure S14. The levels of typical cytokines associated with immune activation in the tumor microenvironment. (A-D) The contents of interferon (IFN)-γ (A), tumor necrosis factor (TNF)-α (B), interleukin (IL)-10 (C), and IL-12p40 (D) in tumor homogenates were determined by ELISA. Data are presented as the mean ± SEM (n = 7).
Figure S15. Safety profiles of CLP nanoparticles in 4T1 tumor-bearing mice after intratumoral injection. (A) Changes in body weight during 15 days of treatment with CLP nanoparticles at 3.25 mg/kg (CLP-L) or 6.5 mg/kg (CLP-H) of Ce6 every 3 days. (B) The organ index values of major organs isolated at day 17 after mice were euthanized. Data are presented as the mean ± SEM (n = 7).
Figure S16. H&E-stained histological sections of major organs after different treatments. Scale bars, 200 μm.
Figure S17. Preliminary chronic toxicity evaluations of CLP nanoparticles in mice. (A) Changes in body weight during 30 days of treatment with saline or CLP nanoparticles every 3 days by intravenous injection. (B) The organ index values of major organs isolated at day 30 after mice were euthanized. (C-F) Complete blood count of representative hematological parameters including WBC (C), RBC (D), HGB (E), and PLT (F). (G-J) Plasma levels of ALT (G), AST (H), CREA (I), and UREA (J) after different treatments. The dose was 25 or 50 mg/kg of Ce6 for the groups CLP-25 and CLP-50, respectively. Data are presented as the mean ± SEM (A, n = 10; B-J, n = 5).
Figure S18. H&E-stained histological sections of major organs from mice subjected to different treatments. After intravenous injection of saline or CLP nanoparticles every three days for 30 days, mice were euthanized for analyses. No significant abnormal changes were observed in CLP-treated groups. Scale bars, 200 μm.
Figure S19. H&E-stained histological sections of different lymphatic systems. Compared to the saline group, no discernable injury was observed in different lymphatic tissues from mice treated with CLP nanoparticles. Scale bars, 200 μm.