Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Layer-by-layered construction of oxygen-generating photo-responsive nanomedicine for enhanced photothermal and photodynamic combination therapy

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Materials

Gold (III) chloride trihydrate ($HAuCl_4$), hydrogen peroxide (H_2O_2), Polyethylenimine MW 25,000 (PEI) and acridine orange hydrochloride hydrate (AO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hoechst 33342, singlet oxygen sensor green (SOSG) and LysoTracker Green DND-26 were purchased from Thermo Fisher (Waltham, MA, USA). Tris(bipyridine)ruthenium(II) chloride ($[Ru(dpp)_3]Cl_2$, RDPP) and Chlorin e6 (Ce6) were purchased from Cayman Chemical (Ann Arbor, MI, USA). Potassium permanganate ($KMnO_4$) was obtained from VWR (Philadelphia, PA, USA). (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) were purchased from Amresco (Solon, OH, USA). Hyaluronic acid (HA), MW 1000,000 and Hyaluronic acid, MW 40,000 (HA_{40K}) was obtained from LifeCore (Chaska, MN, USA).

Preparation for AMP-Ce6-HA

AMP-Ce6-HA was prepared by layer-by-layer assembly. The core gold nanostar (AuNS) was prepared by mixing $HAuCl_4$ (0.1 M, 20 μL) and HEPES (0.1 M, 10 mL) at room temperature for 24 h. The formed AuNS was centrifuged down at 13,000 rpm for 10 min. The collected AuNS was re-suspended in ddH_2O (5 mL) and then reacted with $KMnO_4$ (5 mg/mL, 50 μL) for 1 h. PEI (50 mg/mL, 100 μL) was added to the reaction mixture and reacted for additional 2 h. For thicker MnO_2 coating experiment, a higher amount of $KMnO_4$ (5 mg/mL, 250 μL) and PEI (50 mg/mL, 250 μL) was used. The prepared AuNS@MnO_2-PEI (AMP) was collected by centrifugation (13,000 rpm, 10 min). AMP was then suspended in Tris HCl buffer (pH 8.5, 10 mM, 5 mL). A mixture of Ce6 (1 mM, 50 μL) and HA (10 mg/mL, 50 μL) was added to the AMP solution and vortexed for 2 days. The formed AMP-Ce6-HA was also centrifuged at 13,000 rpm for 10 min. The leftover Ce6 in the supernatant was determined by absorbance, then the Ce6 loading ratio was determined. The obtained AMP-Ce6-HA was res-suspended in phosphate buffer (10 mM, pH 8.5) and stored at 4 °C.

Characterization of AuNS, AMP and AMP-Ce6-HA

Transmission electronic microscope (TEM) images were acquired by TEM (JEM-1400, JEOL, Tokyo, Japan) at the voltage of 120 kV. Hydrodynamic sizes were measured by Malvern Zetasizer Nano-S (Malvern, UK). Zeta potentials were characterized by Zeta PALS analyzer (Brookhaven Instruments, Holtsville, NY, USA). Absorption spectra were obtained by UV-Vis Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Photothermal effect was evaluated by 808 nm laser (CivilLaser, NaKu Technology Co., Ltd, Hangzhou, China)

Photothermal effect evaluation for AMP-Ce6-HA

Different concentrations (0, 4, 12, 20 μg/mL) of AMP-Ce6-HA solution (1 mL) were irradiated by 808 nm laser with a light density of 3.0 W/cm^2 to investigate AMP-Ce6-HA concentration-dependent photothermal effect. Similarly, the AMP-Ce6-HA solution (20 μg/mL, 1 mL) was irradiated by different densities of laser power (0.5, 1.0, 2.0, 3.0 W/cm^2) to evaluate the laser power-dependent photothermal effect. During the irradiation, the solution temperatures were detected by the thermometer (Traceable, VWR, New York, NY, USA) and recorded every 30 s.

pH- and H_2O_2-responsive AMP-Ce6-HA decomposition

AMP-Ce6-HA decomposition was evaluated by monitoring the absorbance of 380 nm at different time points. AMP-Ce6-HA (0.8 mg/mL, 10 μL) was added into pH 7.4, pH 6.5 or pH 5.0 PB buffers (100 μL) to study the pH-responsive decomposition. In order to measure the H_2O_2-responsive decomposition, AMP-

Ce6-HA (0.8 mg/mL, 10 μL) was dissolved into PB buffers (pH 6.5, 100 μL) with different H_2O_2 concentrations (0, 10, 50, 100 μM). Then, the absorbance intensities at 380 nm were recorded at corresponding time points by plate reader (Infinite M1000 Pro, Tecan, Switzerland).

Singlet oxygen detection

Singlet oxygen was detected by SOSG prober. For pH-mediated singlet oxygen generation, AMP-Ce6-HA (0.8 mg/mL, 10 μL) with SOSG (5 μM) was in PB buffers (100 μL) of different pH (pH 7.4, 6.5 and 5.0). To detect the H_2O_2-associated singlet oxygen, AMP-Ce6-HA (0.8 mg/mL, 10 μL) was dissolved into SOSG prober-included (5 μM) PB buffers (pH 6.5, 100 μL) with different concentrations of H_2O_2 (0, 10, 50 and 100 μM). After irradiating these samples for corresponding time, fluorescence intensities of SOSG prober were read by plate reader (ex: 504 nm, em: 520 nm).

Cell uptake for AMP-Ce6-HA

AMP-Ce6-HA (0.8 mg/mL, 2.5 μL) was co-incubated with MDA-MB-231 cells in DMEM+FBS medium (100 μL) for 24 h, 8 h or 4 h, respectively. Free hyaluronic acid competition experiment was performed to confirm the target ability. The cells were pre-treated by HA_{40K} (1 mg/mL) for 2 h, then AMP-Ce6-HA (0.8 mg/mL, 2.5 μL) was added into the medium (100 μL) and co-cultured for another 24 h. Finally, all fluorescence images were acquired at Chlorin e6 channel (ex: 628/40, em: 692/40) by EVOS microscopy.

Intracellular localization AMP-Ce6-HA

MDA-MB-231 cells (100 μL medium per well) were co-incubated with AMP-Ce6-HA (0.8 mg/mL, 2.5 μL) for 24 h. Then, lyso-tracker green (final concentration: 1 μM) was added into the medium and incubated for 1 h, followed by 2 min incubation with Hoechst (final concentration: 0.1 μg/mL). Finally, the fluorescence images were obtained at DAPI channel (ex: 357/44, em: 447/60), GFP channel (ex: 470/22, em: 510/42), and CY5 channel (ex: 628/40, em: 692/40) for nucleus, lysosome and Ce6, respectively.

Phototherapy for MDA-MB-231 cancer cells

For PDT, MDA-MB-231 cells were seeded into 96 well plate (12,000 cells/well) and cultured for 24 h. Different concentrations of AMP-Ce6-HA (final concentration: 0, 4, 8, 12, 16, 20 μg/mL) or Ce6 (final concentration: 0, 0.25, 0.5, 0.75, 1.0, 1.25 μM) was added and incubated for 4, 8 or 24 h. The cells were washed and then irradiated by light (650 nm, 30 mW/cm^2) for 90 s. For PTT, the cells were co-incubated with AMP-Ce6-HA (0, 4, 12, or 20 μg/mL) for 4, 8 or 24 h. The cells were washed and then irradiated with 808 nm laser (3 W/cm^2) for 10 min. For combination of PDT and PTT, the cells pre-treated by AMP-Ce6-HA (12 μg/mL) for 24 h were first irradiated by 650 nm light (30 mW/cm^2) for 90 s, and then by 808 nm light (3 W/cm^2) for 10 min. The cell viability was evaluated 16 hours later by CCK-8 viability kit.

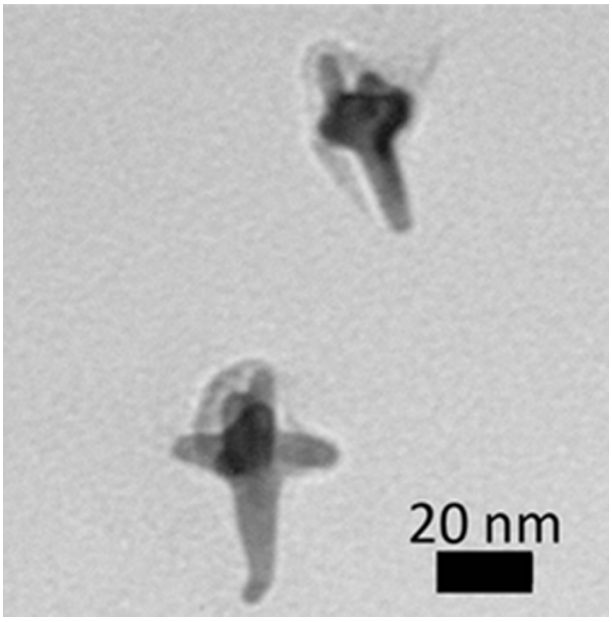

Fig. S1. TEM image of AMP prepared with $KMnO_4$ (0.05 mg/mL) and PEI (1.0 mg/mL).

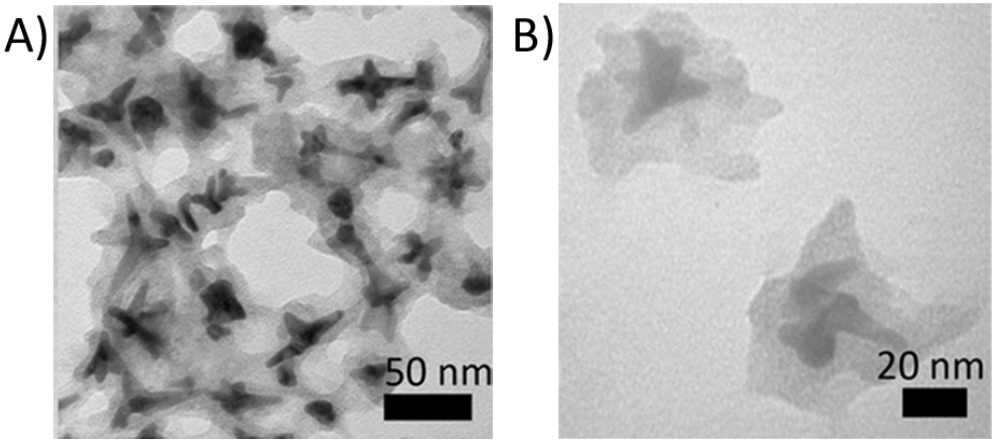

Fig. S2. TEM image of AMP prepared with high concentrations of $KMnO_4$ (0.25 mg/mL) and PEI (2.5 mg/mL).

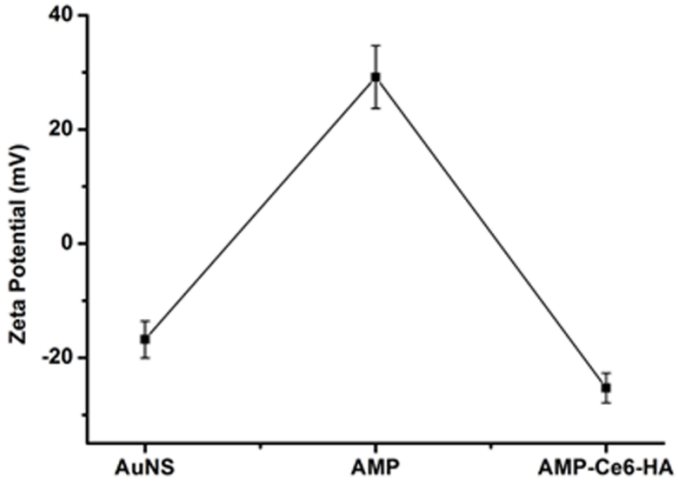

Fig. S3. Zeta potentials of AuNS, AMP and AMP-Ce6-HA.

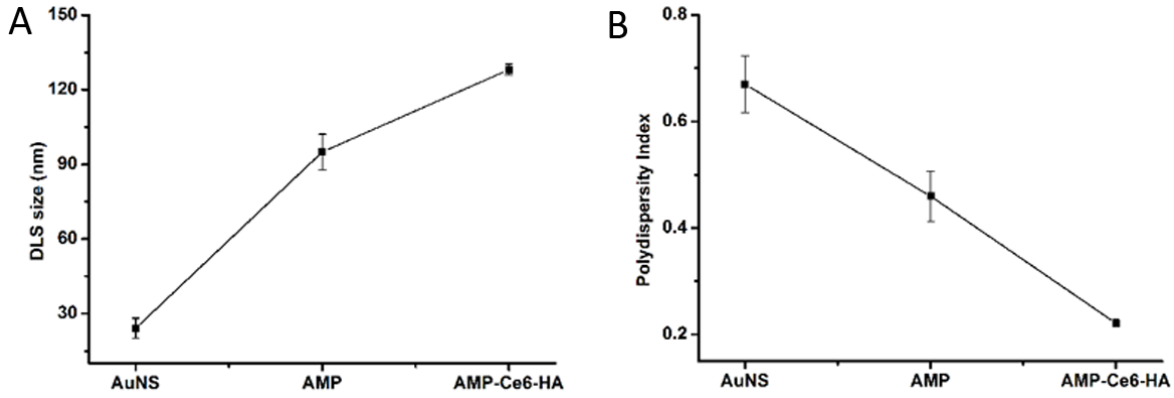

Fig. S4. DLS sizes (A) and PDIs (B) of AuNS, AMP and AMP-Ce6-HA.

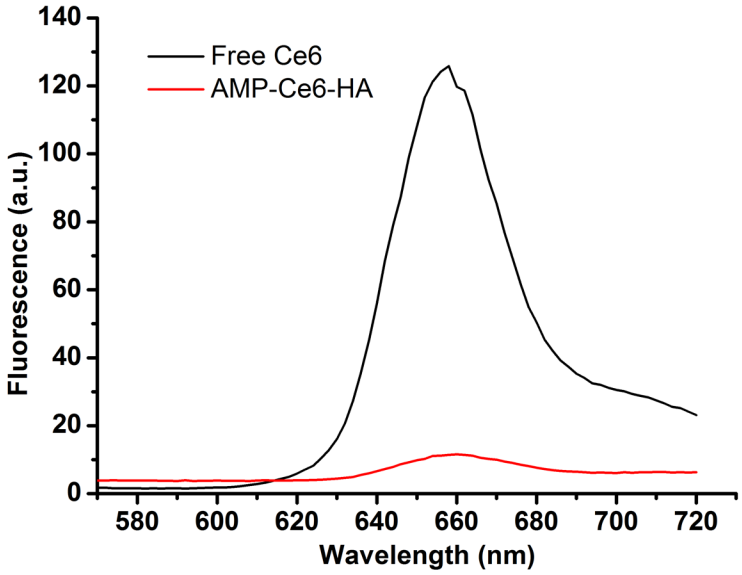

Fig. S5. Fluorescence of spectra of Ce6 (1 μM) before and after being loaded into nanoparticles.

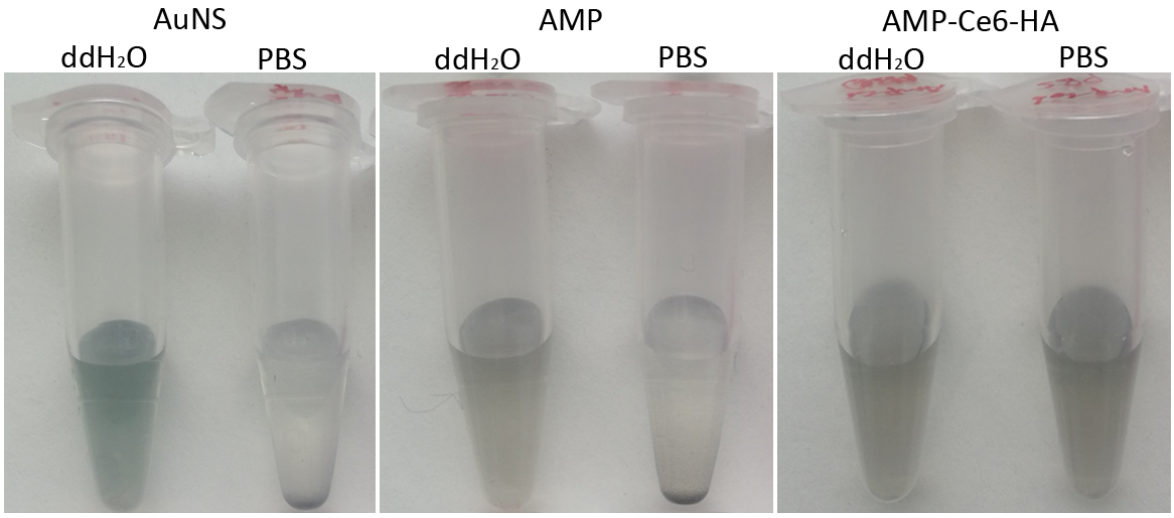

Fig. S6. Digital images of AuNS, AMP and AMP-Ce6-HA in ddH_2O or PBS for 24 h. Note the aggregation of AuNS and AMP in PBS.

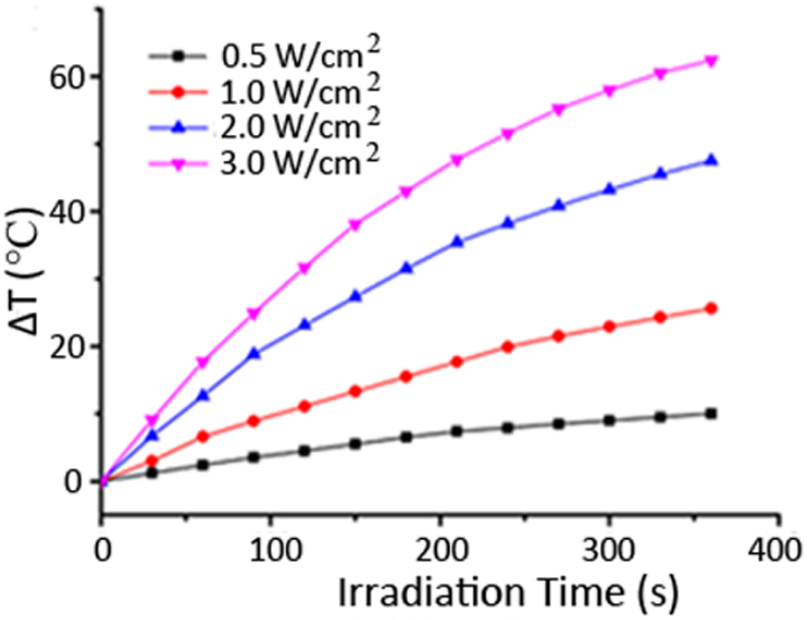

Fig. S7. Laser power-dependent temperature change at 20 μg/mL of AMP-Ce6-HA aqueous solution.

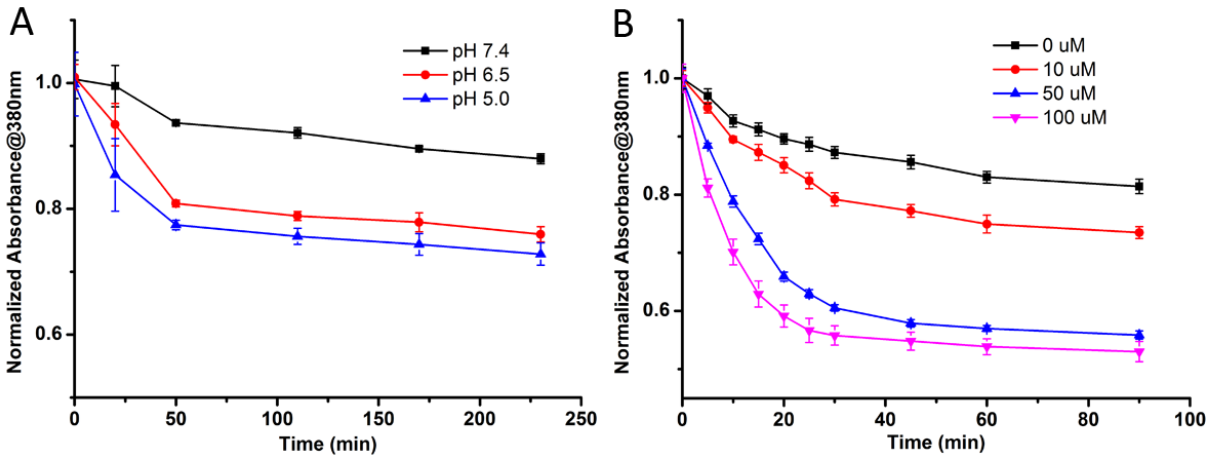

Fig. S8. A) Normalized absorption change at 380 nm of AMP-Ce6-HA in pH 7.4, 6.5 or 5.0 buffer for different time. B) Normalized absorption change at 380 nm of AMP-Ce6-HA in pH 6.5 buffer containing different concentrations of H_2O_2.

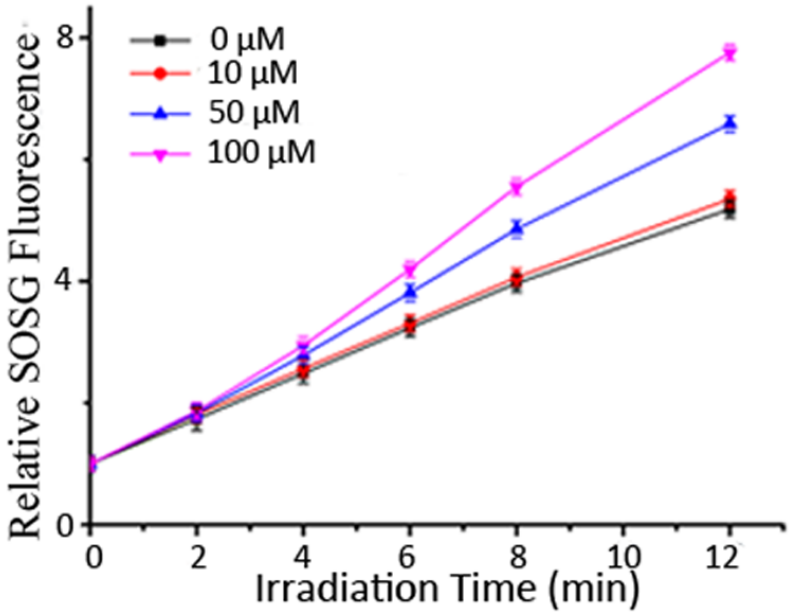

Fig. S9. Relative SOSG fluorescence of AMP-Ce6-HA with H_2O_2 (0 - 100 μM, pH 6.5) after irradiation (650 nm light, 30 mW/cm^2).

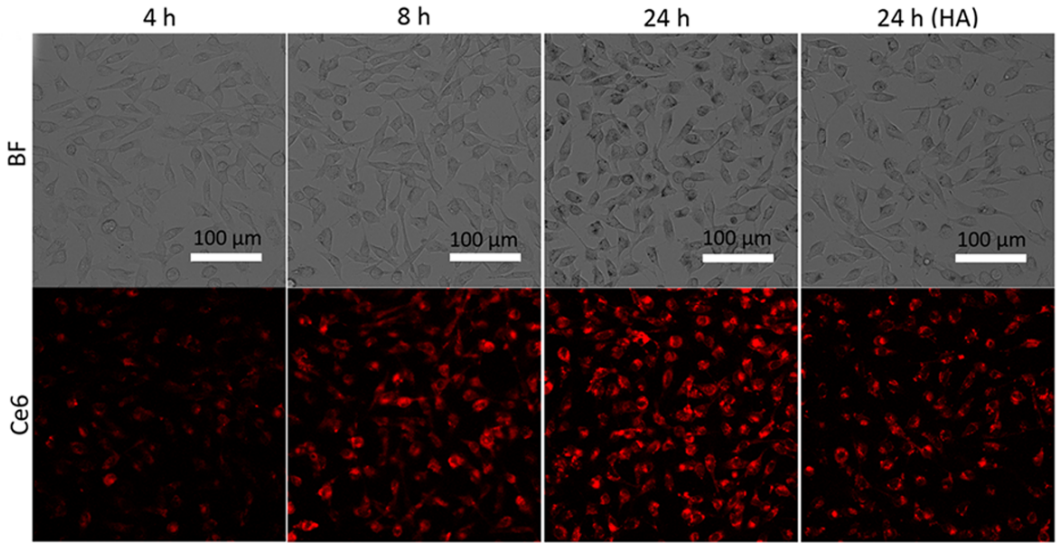

Fig. S10. Bright field (BF) and fluorescence (Ce6) images of MDA-MB-231 cells with AMP-Ce6-HA (20 μg/mL) for 4 h, 8 h or 24 h without free HA, as well as 24 h with free HA addition (1 mg/mL).

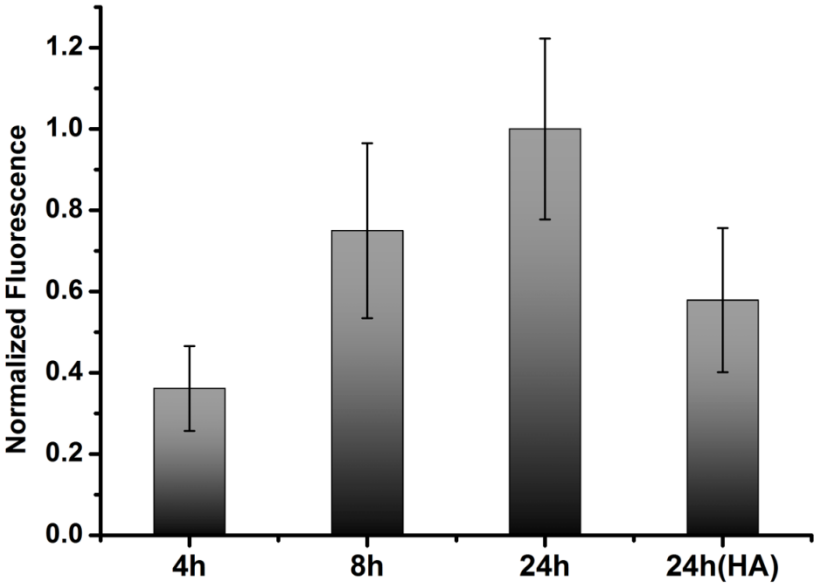

Fig. S11. Normalized Intracellular fluorescence of MDA-MB-231 cells after being incubated with AMP-Ce6-HA at different time points in HA-free or HA-supplemented (HA concentration: 1 mg/mL) medium.

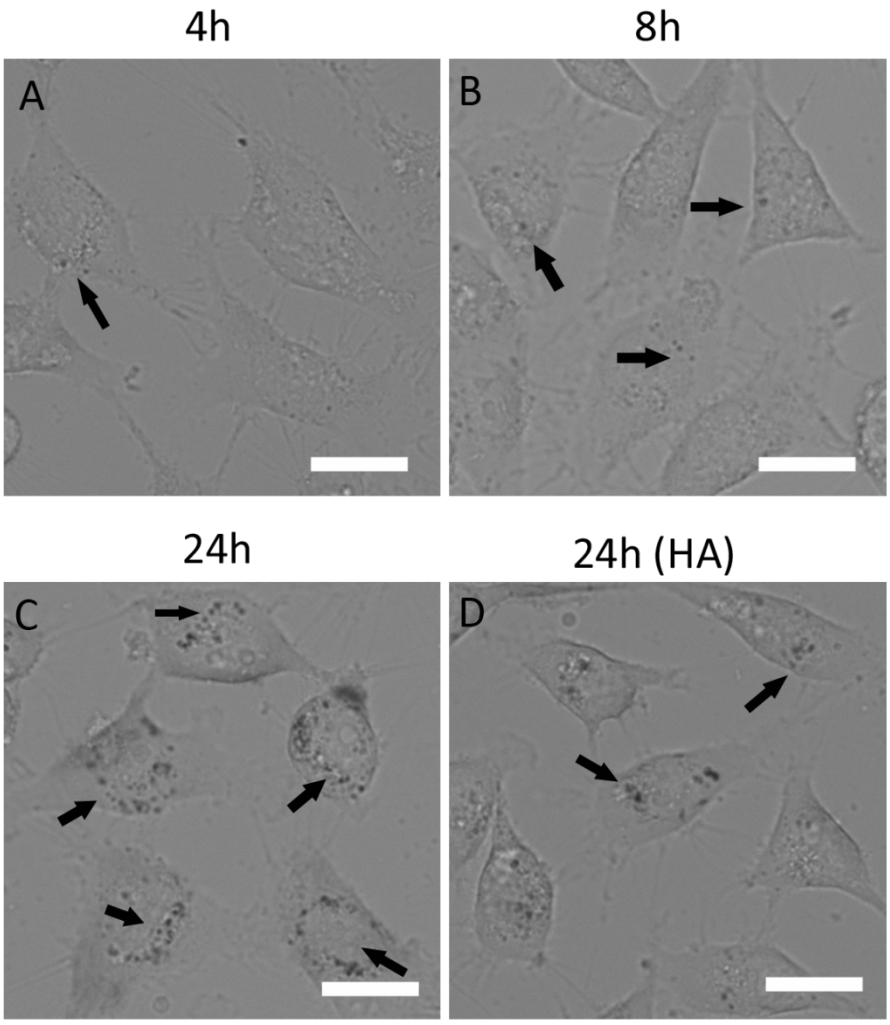

Fig. S12. Bright field (BF) images of MDA-MB-231 cells after being incubated with AMP-Ce6-HA (20 μg/mL) for 4 h, 8 h or 24 h without HA, as well as 24 h with excess of free HA (1 mg/mL). Scale bar is 20 μm.

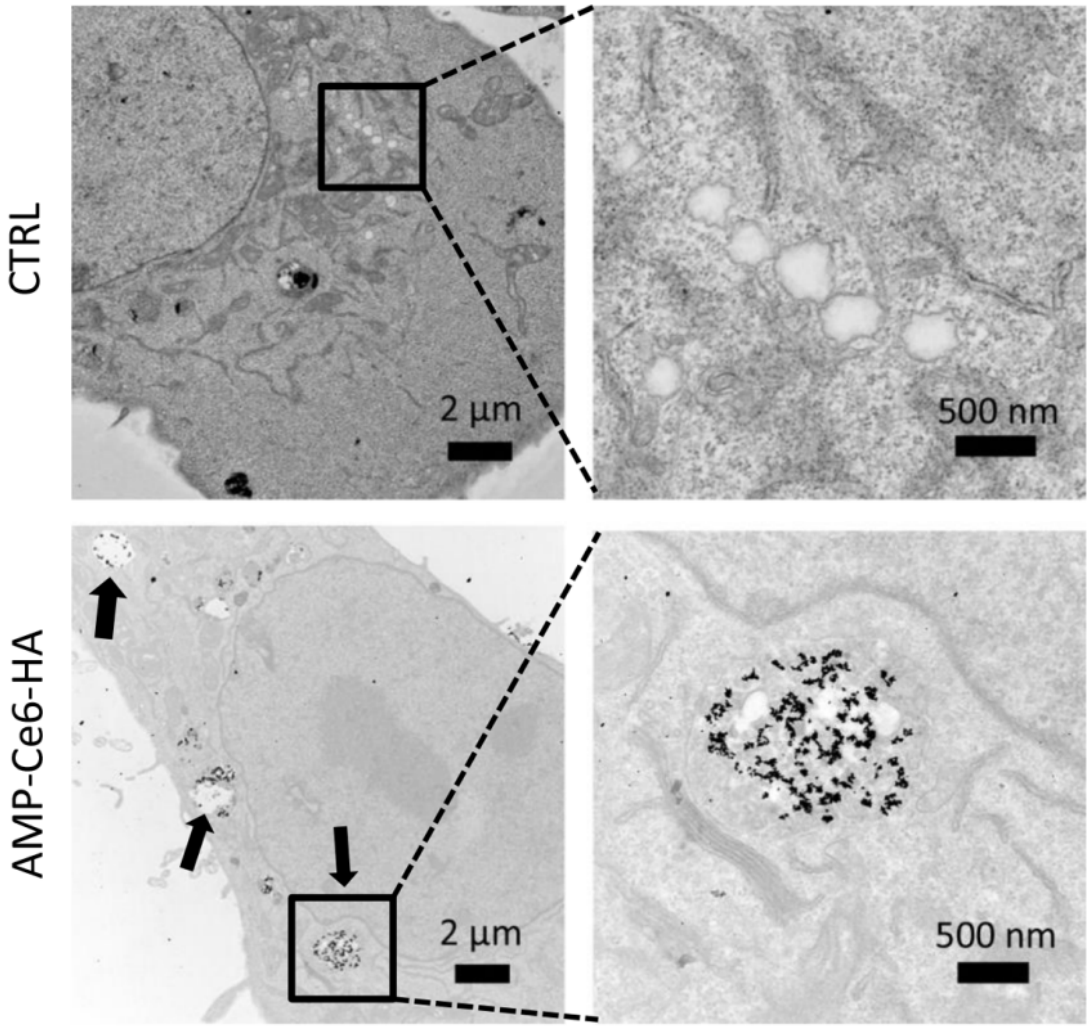

Fig. S13. TEM images for MDA-MB-231 cells incubated with or without AMP-Ce6-HA for 24 h.

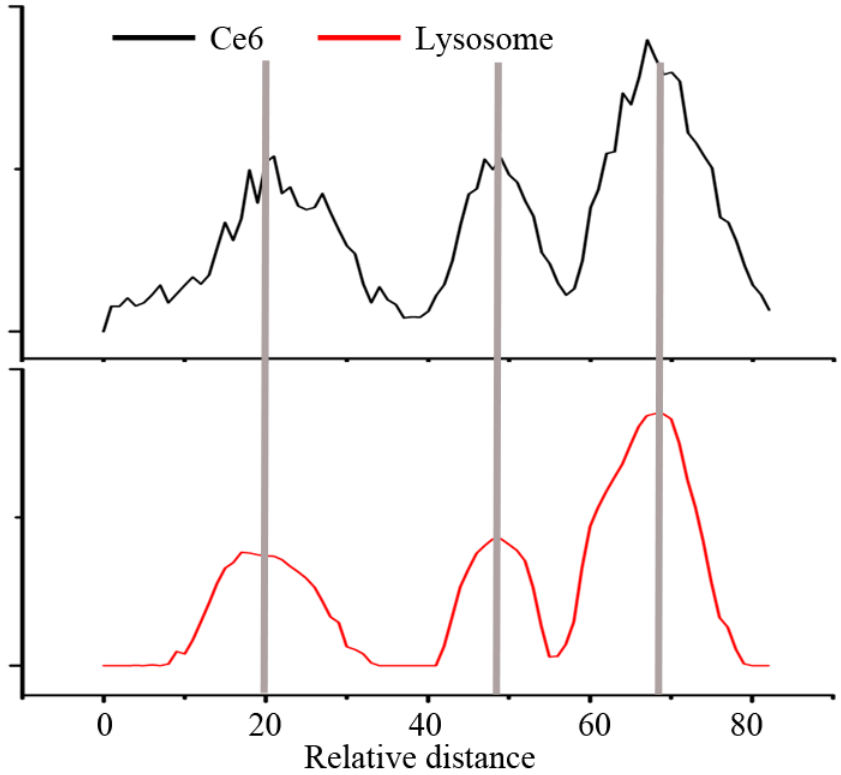

Fig. S14. Spatial fluorescence intensity of Ce6 and Lysosome (AO) image across the marked line in Figure 3C.

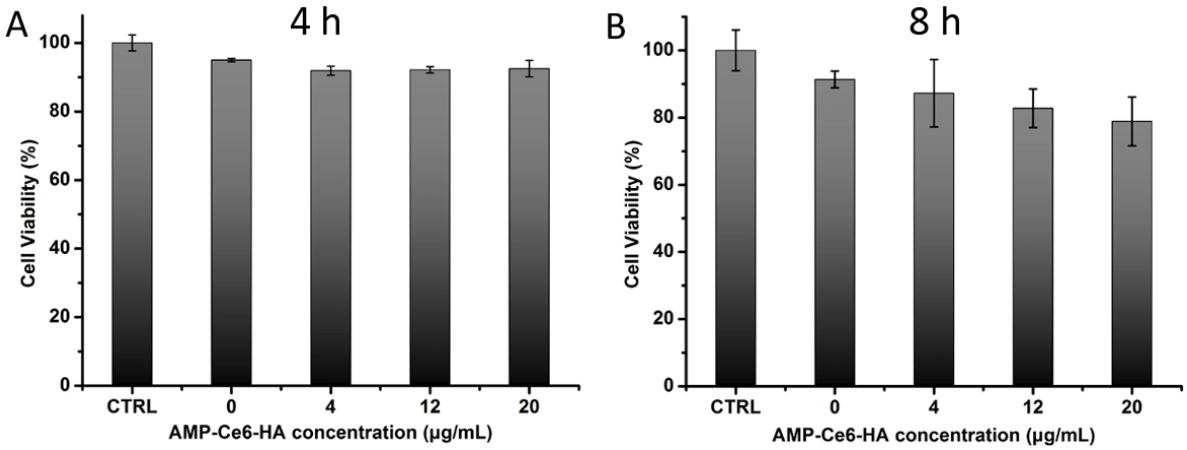

Fig. S15. PTT effect of AMP-Ce6-HA in cells viability. MDA-MB-231 cells were incubated with different concentrations of AMP-Ce6-HA for A) 4 h and B) 8 h, and then irradiated by 808 nm light (3 W/cm^2) for 10 min.

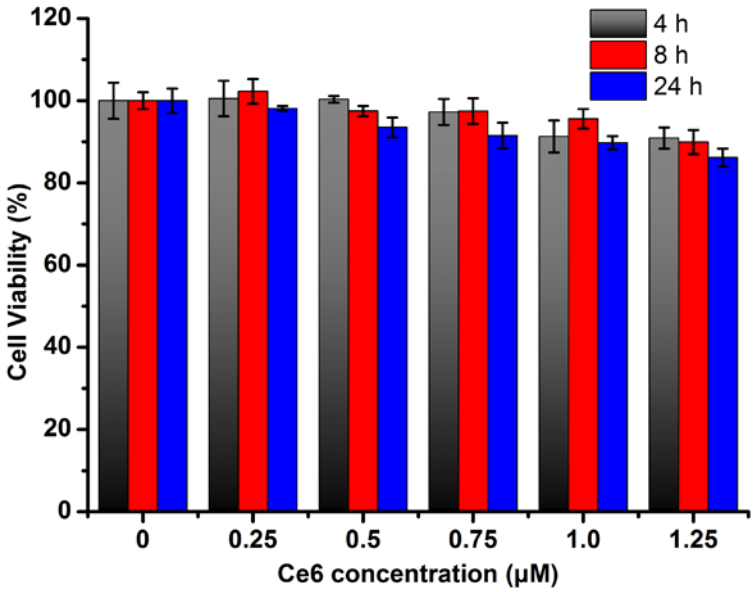

Fig. S16. PDT effect of free Ce6 in cells viability. MDA-MB-231 cells were incubated with Ce6 of different concentrations for 4 h, 8 h or 24 h, and then irradiated by 650 nm light (30 mW/cm^2) for 90 s.