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Supporting Information

In Situ Formation of Large Pore Silica-MnO_2 Nanocomposites with H^+/H_2O_2 Sensitivity for O_2-Elevated Photodynamic Therapy and Potential MR Imaging

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Apparatus and characterization. Transmission electron microscopy (TEM) measurement was operated on a JEM-200CX transmission electron microscope (JEOL, Japan) with an accelerating voltage of 200 kV. Scanning electron microscope (SEM) images were acquired from a Hitachi S-4800 scanning electron microscope (Hitachi, Japan). Scanning transmission electron microscope (STEM) images and energy-dispersive X-ray (EDX) mapping were obtained from a field emission scanning electron microscope Zeiss Ultra Plus (Carl Zeiss, Germany). X-ray photoelectron spectroscopy (XPS) measurement was performed on a PHI 5000 Versa Probe spectrophotometer (UlVAC-PHI, Japan). UV-Vis spectra were recorded using a UV-3600 spectrophotometer (Shimadzu, Japan). Zeta potential was measured on a nano-z zeta potential analyzer (Malvern Instruments, USA). Fourier transform infrared spectroscopy (FTIR) spectra were acquired from a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, USA). Dynamic light scattering (DLS) measurement was carried out using a 90 Plus Nanoparticle Size Analyzer (Brookhaven, USA). Nitrogen adsorption-desorption isotherm was obtained from a Micromeritics ASAP 2020M automated sorption analyzer (Micromeritics, Co., USA). Prior to measurement, the sample was degassed at 100 °C for 6 h. The Brunauer-Emmett-Teller (BET) specific surface area was calculated according to adsorption data in low pressure and the corresponding pore diameter was estimated using the Barrett-Joyner-Halenda (BJH) method. The Mn content was determined by means of inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher, USA). MTT assay was assessed by measuring the absorbance at 490 nm on a Varioskan Flash instrument (Thermo Scientific, USA). Confocal laser scanning microscopy (CLSM) characterization was performed on a Leica TCS SP5 confocal microscope (Leica Microsystems, Germany). Flow cytometry analysis was conducted on a Cytomics FC 500 MCL instrument (Beckman Coulter, USA). *In vitro* MRI performance was tested on a 3.0 T human magnetic resonance scanner (Signa, USA).

Materials. Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium tosylate (CTATos), $KMnO_4$, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and (3-Glycidyloxypropyl)trimethoxysilane (3-GPS) were purchased from Sigma-Aldrich (USA). Triethanolamine (TEA), PEI (M.W.: 600 Da), Ethylenediamine and N-hydroxysulfosuccinimide sodium salt (NHS) were obtained from Aladdin (Shanghai, China). Chlorin e6 (Ce6) was bought from J&K chemical company (Beijing, China). Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride ($[Ru(dpp)_3]Cl_2$) was obtained from Alfa Aesar (MA, USA). The aptamer AS1411-COOH was supplied by Sangon Biological Engineering Technology & Services Co. (Shanghai, China) and its sequence is as follows: 5'-COOH-TTTTTTGGTGGTGGTGGTTGTGGTGGTGGTGG-3'. Ultrapure water form a Millipore Milli-Q system (USA) was used throughout all experiments.

Preparation of LPMSNs-PEI. LPMSNs-PEI were synthesized following reported methods with slight modifications.[1] The synthesis process was divided into three steps: (1) preparation of LPMSNs-OH; (2) epoxysilane functionalization and (3) PEI grafting. Firstly, LPMSNs-OH were synthesized as follows: a mixed solution of 0.96 g CTATos, 0.173 g TEA and 50 mL water was

gently stirred at 80 ºC for 1 h. After that, 7.8 mL TEOS was quickly poured into the above aqueous solution. The mixture was kept at 80 ºC for 2 h with a high stirring speed of 1000 rpm. The synthetic product (LPMSNs-OH) was isolated by centrifugation and washed several times with water and ethanol. To remove the surfactant CTATos, the precipitate was resuspended in HCl-ethanol (v/v = 3:20) mixed solvent and then subjected to heating reflux at 75 ºC for 24 h. The removal process was repeated twice for efficient surfactant remove. After centrifugation and washing with ethanol, the white precipitate was dispersed in ethanol and stored at 4 ºC. Secondly, epoxysilane functionalization was realized *via* alcoholysis reaction. Specially, the extracted LPMSNs-OH (100 mg) were refluxed in 100 mL ethanol which contained 3-GPS (120 μL) at 80 ºC for 24 h. The resultant product (LPMSNs-epoxy) was centrifuged, washed and dispersed in water. Thirdly, for PEI grafting, 8 mg LPMSNs-epoxy and 2 mg PEI were added into 6 mL carbonate buffer (50 mM, pH 9.5). After 24 h of stirring, 3 μL ethylenediamine was added and the mixture was stirred for another 5 h. Finally, LPMSNs-PEI were collected by centrifugation and washed thrice with water and dispersed into water for further use.

***In situ* MnO_2 growth for LPMSNs-MnO_2.** 2 mg LPMSNs-PEI were dispersed into 1 mL water and sonicated for uniform dispersion. Then, 140 μL 1 mg/mL $KMnO_4$ was added dropwise and brown LPMSNs-MnO_2 were obtained.

Synthesis of MnO_2 NPs. MnO_2 NPs were fabricated using short-chain PEI as the reducing agent. Briefly, 8 mL of $KMnO_4$ aqueous solution (10 mg mL^{-1}) was added dropwise into 15 mL of PEI (16 mg mL^{-1}) under rapid stirring and the mixture was kept stirring for another 20 min. Later, brown MnO_2 NPs were precipitated using acetone and were isolated by centrifugation. After drying in vacuum, the pellets were subjected to X-ray diffraction measurement.

Ce6 and AS1411 functionalization for AS1411/Ce6-LPMSNS-MnO_2. Both Ce6 and AS1411 were covalently linked to LPMSNs-MnO_2 through EDC/NHS coupling reaction. In the case of Ce6 conjugation, 1 mg Ce6, 1 mg EDC and 1.5 mg NHS were dissolved in 1.2 mL DMF for 30 min to activate the carboxyl group of Ce6. The activated Ce6 was then added into LPMSNs-MnO_2 aqueous suspension (0.3 mL, 10 mg mL^{-1}) and reacted for 6 h. The unreacted Ce6 was removed by centrifugation and washing to obtain Ce6-LPMSNs-MnO_2. As for AS1411 modification, 15 μL AS1411-COOH (100 μM) aqueous solution was mixed with EDC (3 μL, 10 mg mL^{-1}) and NHS (3 μL, 10 mg mL^{-1}), and the total volume was adjusted to 100 μL with water. After 30 min of activation, Ce6-LPMSNS-MnO_2 (100 μL, 1 mg mL^{-1}) in DMF was added to the mixture and then gently shaken for 4 h. The AS1411/Ce6-LPMSNS-MnO_2 was then collected by centrifugation and several washing.

Singlet oxygen detection. Singlet oxygen level was measured by the decline of the DPBF absorption at 416 nm following a reported method.[2, 3] AS1411/Ce6-LPMSNs-MnO_2 were first dispersed in 250 μM H_2O_2-containing PBS (10 mM, pH 6.5). After 30 min of standing, the mixture was added into 10 μg mL^{-1} DPBF-containing DMSO solution and exposed to laser irradiation (660

nm, 0.8 W cm^{-2}). At the certain points, the absorbance of the solution was recorded by the UV-Vis spectrophotometer.

Cell Culture and *in vitro* cytotoxicity assay. NIH 3T3 mouse fibroblast (NIH 3T3) and Human cervical carcinoma cells (HeLa) cells were supplied by KeyGen Biotech Co. Ltd. (Nanjing, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) with the addition of 10% fetal bovine serum (FBS), 100 μg mL^{-1} streptomycin and 100 μg mL^{-1} penicillin. The cells were then placed in a humidified incubator at 37 °C with a supply of 5% CO_2 atmosphere.

MTT experiments were employed to evaluate the biocompatibility and phototoxicity of the nanomaterials. HeLa cells were seeded in 96-well plates at a density of 5×10^3 cells per well for 24 h, followed by incubation with different nanomaterials for another 24 h. In the study of phototoxicity, a 660 nm laser with a power density of 100 mW cm^{-2} was applied to irradiate the cells for 10 min. The cells were further cultured for 24 h and then subjected to MTT assay. Briefly, 10 μL of 5 mg mL^{-1} MTT was added to each well. 4 h later, the medium was substituted with 100 μL of dimethyl sulfoxide (DMSO) to dissolve the formed formazan crystals. The absorbance at 490 nm was then recorded by a Varioskan Flash instrument to assess the viability of the cells.

Confocal fluorescence imaging. For the study of intracellular O_2 generation from AS1411/Ce6-LPMSNs-MnO_2, HeLa cells were pretreated with 5 μM $[Ru(dpp)_3]Cl_2$ for 4 h, and subsequently cultured with the nanomaterials for 0 h, 4 h, 18 h and 24 h, respectively.[4] At predetermined points, the cells were rinsed three times with PBS and prepared for imaging. The cells were excited at 488 nm, and the fluorescence emission (600-650 nm) was then collected by a confocal microscope.

In the experiments of detecting the intracellular ROS level, HeLa cells were treated with 20 μg mL^{-1} AS1411/Ce6-LPMSNs-MnO_2 for 24 h. After rinsing with PBS, the cells were further incubated with 10 μM DCFH-DA for 20 min. Then, the cells were rinsed with PBS and were exposed to laser irradiation (100 mW cm^{-2}, 10 min). For confocal imaging, the excitation was 488 nm and the emission range was 510-560 nm.

Flow cytometry analysis. Flow cytometry experiments were carried out to investigate the cellular uptake. Cells were seeded in 6-well plates with a density of 2×10^5 cells per well for 24 h. Then, the medium was replaced with fresh one containing nanomaterials. After culture for 4 h, the cells were harvested, rinsed by PBS, resuspended, and measured by flow cytometry.

Relaxivity measurements of the nanocomplexes. The *in vitro* T_1 MRI performance of AS1411/Ce6-LPMSNs-MnO_2 was studied on a 3.0 T system. Serial concentrations of the nanomaterials at pH 7.4 or acidic H_2O_2 (pH 6.5) were prepared for MR scanning. The corresponding parameters were listed as follows: matrices = 384 × 256, TR/TE = 425/16.5 ms, field of view (FOV) = 14 cm × 14 cm and slice thickness = 2.0 mm.

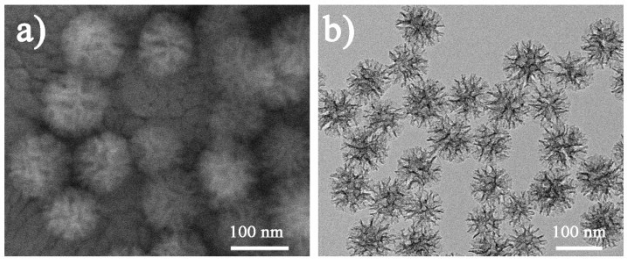


Fig. S1 (a) SEM and (b) TEM images of LPMSNs-PEI.

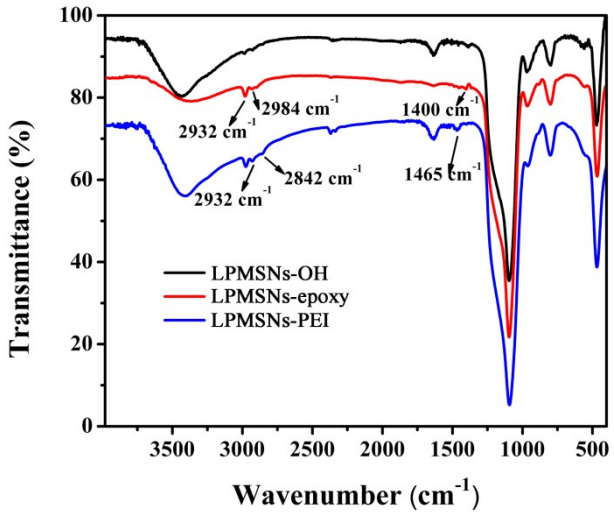


Fig. S2 FTIR spectra of LPMSNs-OH, LPMSNs-epoxy and LPMSNs-PEI.

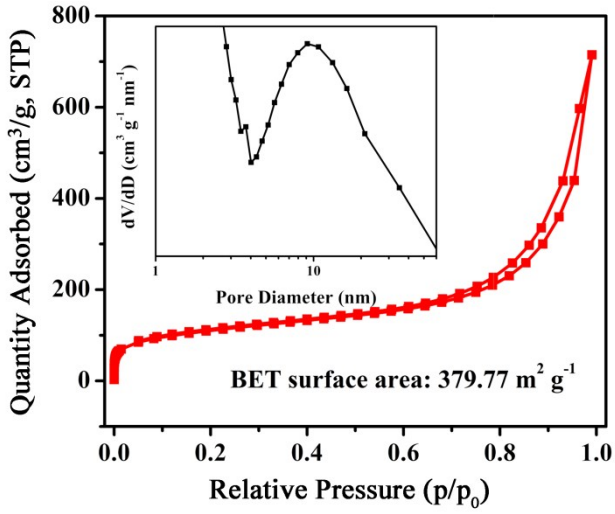


Fig. S3 N_2 adsorption-desorption isotherm of LPMSNs-PEI. Inset is the corresponding pore size distribution.

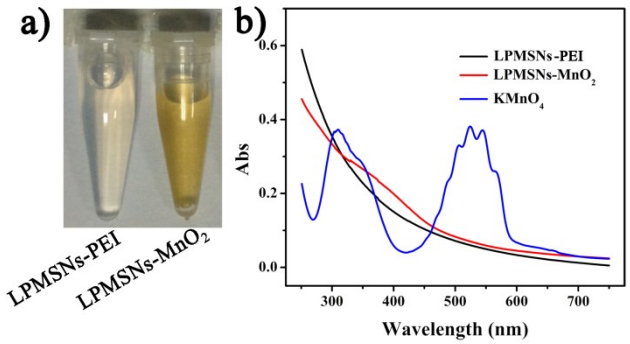


Fig. S4 (a) The photograph of LPMSNs-PEI and LPMSNs-MnO_2. (b) UV-Vis spectra of LPMSNs-PEI, LPMSNs-MnO_2 and $KMnO_4$.

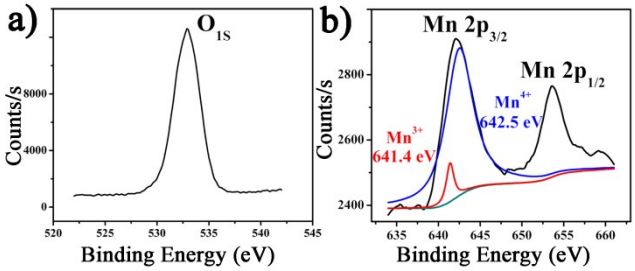


Fig. S5 XPS spectra of LPMSNs-MnO_2.

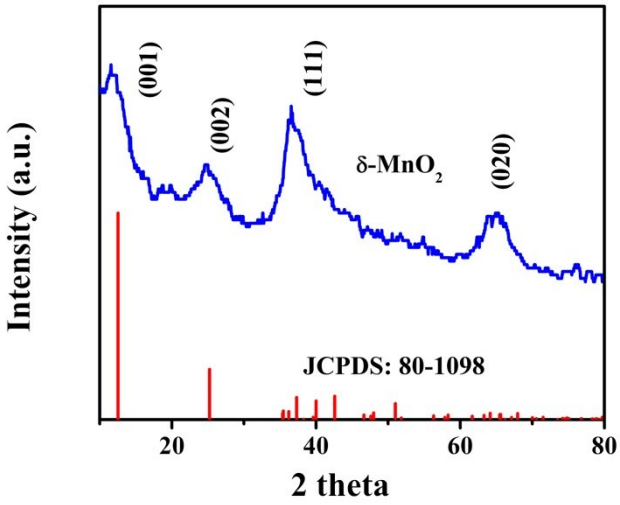


Fig. S6 X-ray diffraction pattern for MnO_2 NPs which were synthesized using PEI as the reducing agent.

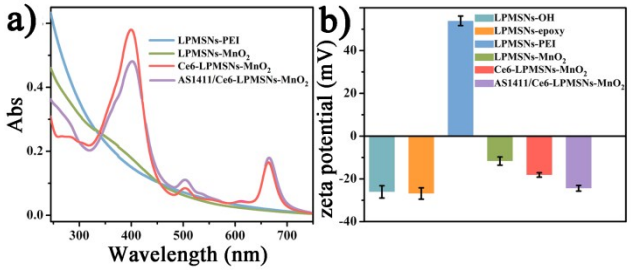


Fig. S7 Evolution of (a) absorption spectra and (b) zeta potential during the assembly processes.

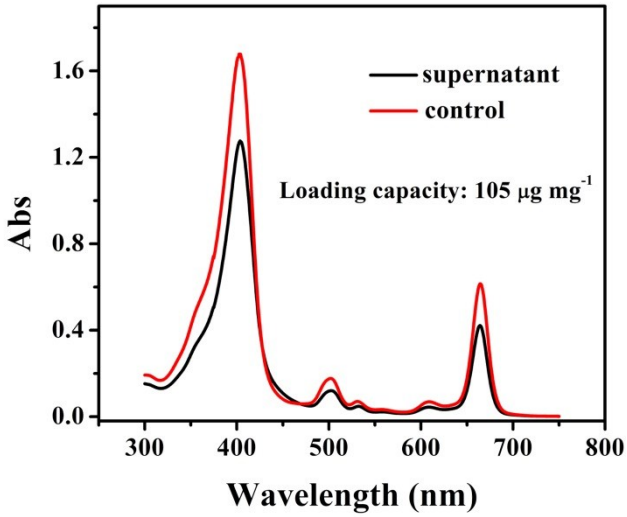
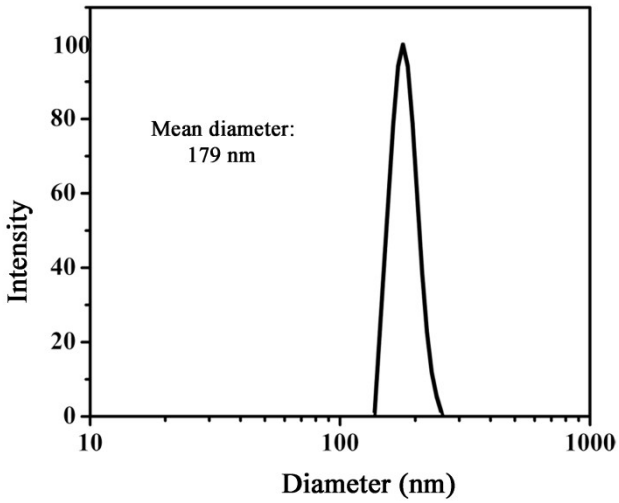


Fig. S8 UV-Vis spectra for total and supernatant Ce6 molecules. The amounts of LPMSNs-MnO_2 and Ce6 used here were 1 mg and 0.33 mg, respectively. And the loading capacity was calculated by comparing the absorbance at 665 nm of the total and unreacted Ce6 molecules.



. **Fig. S9** The diameter of the AS1411/Ce6-LPMSNs-MnO_2.

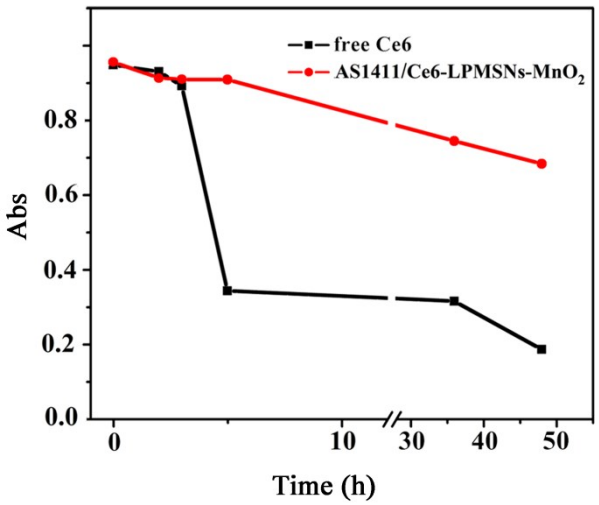


Fig. S10 The absorbance of free Ce6 and AS1411/Ce6-LPMSNs-MnO_2.

$$MnO_2 + H_2O_2 + 2H^+ \rightarrow O_2\uparrow + Mn^{2+} + 2H_2O$$

Fig. S11 *In vitro* reactivity of MnO_2 towards H^+/H_2O_2.

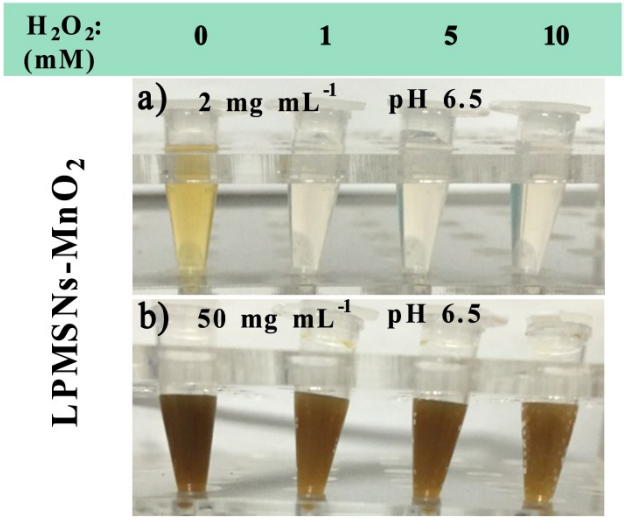


Fig. S12 Photos of LPMSNs-MnO_2 dispersed in pH 6.5 buffers with increasing concentrations of H_2O_2. The concentration of LPMSNs-MnO_2 is (a) 2 mg mL^{-1} and (b) 50 mg mL^{-1}.

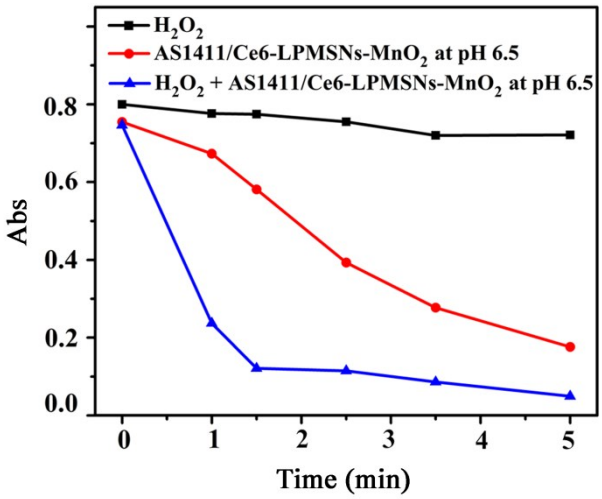


Fig. S13 Time-dependent decay of DPBF absorbance at 416 nm with different treatments after 660 nm irradiation (0.8 W cm^{-2}).

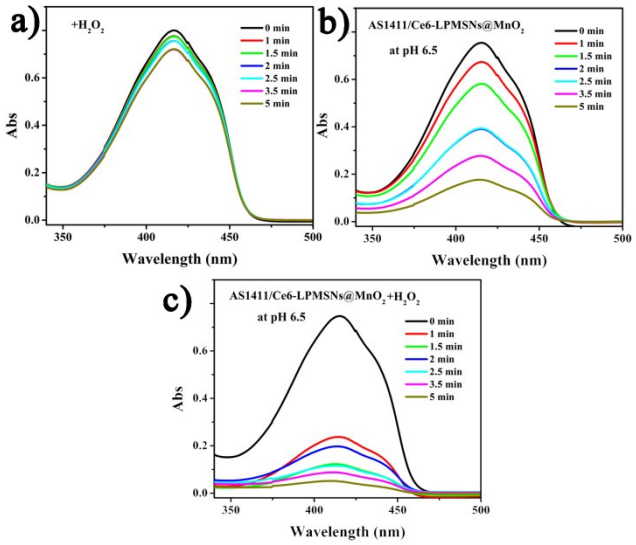


Fig. S14 UV-Vis spectra of DPBF with different treatments under 660 nm irradiation with a power density of 0.8 W cm^{-2}: (a) H_2O_2, (b) AS1411/Ce6-LPMSNs-MnO_2 and (c) AS1411/Ce6-LPMSNs-MnO_2 after incubation with H_2O_2 at pH 6.5.

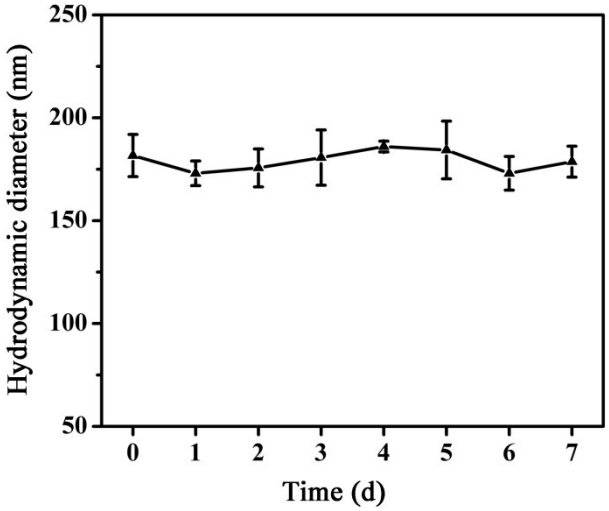


Fig. S15 The diameter change of AS1411/Ce6-LPMSNs-MnO_2 during 7 d incubation in 10% FBS-containing medium.

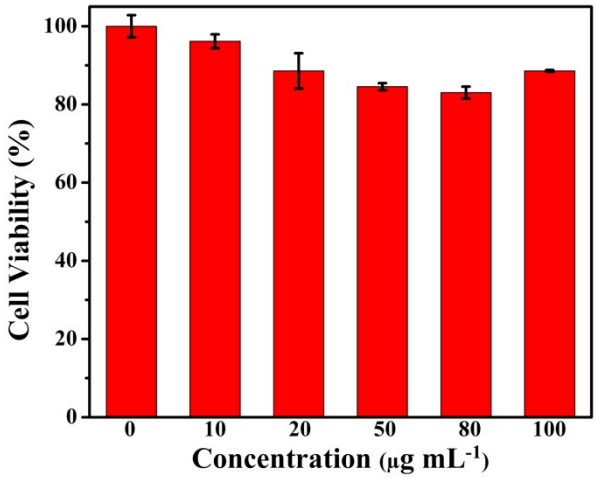


Fig. S16 MTT assay for HeLa cells after 24 h culture with AS1411/Ce6-LPMSNs-MnO_2 in the absence of light.

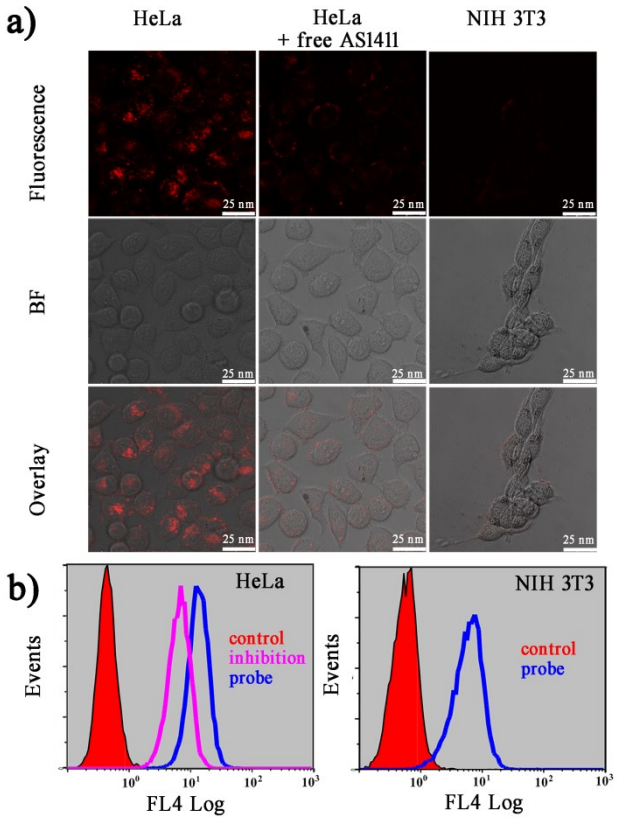


Fig. S17 (a) CLSM images and (b) flow cytometric assay of HeLa and NIH 3T3 cells treated with 20 μg mL^{-1} AS1411/Ce6-LPMSNs-MnO_2 for 4 h. In the inhibition group, HeLa cells were pre-incubated with free AS1411 molecules for 40 min.

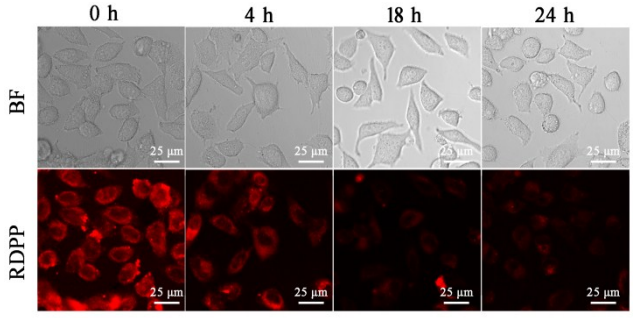


Fig. S18 CLSM images illustrating increased O_2 level within HeLa cells after incubation with AS1411/Ce6-LPMSNs-MnO_2. HeLa cells cultured with 5 μM $[Ru(dpp)_3]Cl_2$ for 4 h, and subsequently incubated with the nanomaterials for 0 h, 4 h, 18 h and 24 h. Scale bar: 25 μm.

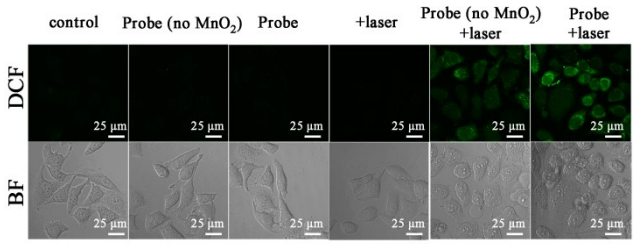


Fig. S19 CLSM images reflecting ROS level within HeLa Cells which were treated with different nanomaterials under laser irradiation. Scale bar: 25 μm.

References

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