

Supporting Information for

**In Vivo Lighted Fluorescence *via* Fenton Reaction: A New
Approach for Imaging of Hydrogen Peroxide in Living Systems**

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Instruments. ^1H NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. Liquid chromatography-mass spectrometry (LC-MS) analyses were performed using an Agilent 1100 HPLC/MSD spectrometer. Transmission electron microscopy (TEM) images were obtained with a JEOL-3010 instrument. Zeta potential experiments and dynamic light scattering (DLS) measurements were performed by ZetaSizer Nanoseries. Fourier transform infrared (FT-IR) spectra were obtained from a TENSOR 27 spectrometer (Bruker Instruments Inc., Germany). Small-angle powder X-ray diffraction patterns (XRD) were obtained in a Scintag XDS-2000 powder diffractometer. Nitrogen adsorption-desorption isotherm was obtained on a Micromeritics ASAP 2010 Sorptometer. All fluorescence measurements were performed on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). Confocal laser scanning microscopy (CLSM) images were obtained on a Fluo ViewTM FV 1000 (Olympus, Japan). MTT assay was obtained on a Benchmark Plus (Bio-Rad Instruments Inc., Japan). *In vivo* images were acquired using an IVIS Imaging System (Lumina xr). The pH was measured by a model 868 pH meter (Orion).

Synthesis of Perylene Tetracarboxylic Acid Diimide Dimmer (PTAD). Perylene dimethylamine (PTCDI) was prepared according to the literature (Wang, B.; Yu, C. *Angew. Chem. Int. Ed.* **2010**, *49*, 1485-1488). Then, perylene dimethylamine (50 mg, 0.089 mmol) was dissolved in 2.5 mL of toluene, then 50 μL of 1,2-dibromoethane (0.577 mmol) was added to the solution and the reaction mixture was refluxed for 5 h under nitrogen atmosphere. Finally, PTAD was gained in 42.6% yield by filtering, washing with ether, followed drying under vacuum. ^1H NMR (400 MHz, DMSO- d_6 , TMS): δ 8.78 (d, $J=8.1$, 8H), 8.46 (d, $J=8.3$, 8H), 6.1-5.62 (m, 8H), 4.1 (d, $J=7.8$, 8H), 2.91-3.06 (m, 12H), 2.33-2.67 (m, 12H), 2.09 (s, 12H); ESI-MS: m/z 575.2 ($\text{M}^{2+}/2$).

Cell Culture. HeLa cells and A549 cells were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/mL 1%

penicillin/gentamicin at 37 °C under 5% CO₂ atmosphere. HEK 293 cells were cultured in DMEM supplemented with only 10% fetal bovine serum at 37 °C under 5% CO₂ atmosphere.

MTT Assays. HeLa cells (2×10^5 cells per well) were dispersed in 96-well microtiter plates and maintained at 37 °C, in 5% CO₂ for 24 h. Then HeLa cells were incubated with the nanosphere (0.1 mg/mL) at different concentrations for 24 h. Next, the cell culture medium was removed, following the addition of MTT solution (5 mg/mL) to each well. After 4 h, the remaining MTT solution was removed, followed by supplementation with 150 µL of DMSO to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader.

Characterization of the Cell-endocytosed Nanosphere by Transmission Electron Microscopy. HeLa cells were treated with the nanosphere (0.1 mg/mL) for 2 h at 37 °C. After washing and centrifugation, the cell pellets were fixed with 2.5% glutaraldehyde in 0.1 M PBS for 2.5 h, dehydrated using an serial concentrations of alcohol (20%, 40%, 60%, 80%, and 100% twice) for 20 min, and then embedded in Araldite resin at 65 °C overnight. A 70 nm section was placed on a TEM grid and stained with saturated uranyl acetate and 0.2% Reynolds lead citrate before TEM imaging.

Animals Culture. Athymic BALB/c nude mice were purchased from the Changsha SLAC Laboratory Animal Co. Ltd. and maintained under pathogen-free conditions. To establish tumor xenografts, 5×10^6 *in vitro*-propagated HeLa cells (in 100 µL of PBS buffer) were injected s.c. into the back of 18 ~ 20 g nude mice. All animal operations were performed in accordance with institutional animal use and care regulations, according to protocol No. SYXK (Xiang) 2008-0001, approved by the Laboratory Animal Center of Hunan. Once the tumors had grown to a volume of $\sim 100 \text{ mm}^3$, they were ready for the *in vivo* imaging. All animals were acclimated to the animal facility for at least 48 h prior to experimentation.

Table S1. Physicochemical properties of MSN, MSN-F_{DNA} and Fc@MSN-F_{DNA}/PTAD.

Particles	Surface area (m ² /g)	Pore Volume (cm ³ /g)	Pore Size (nm)
MSN	1057.8	0.832	3.06
MSN-F _{DNA}	614.5	0.623	2.60
Fc@MSN-F _{DNA} /PTAD	222.3	/	/

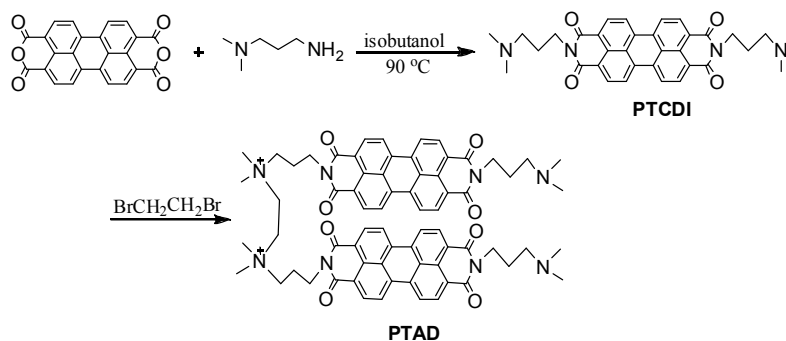


Figure S1. Synthetic route for PTAD.

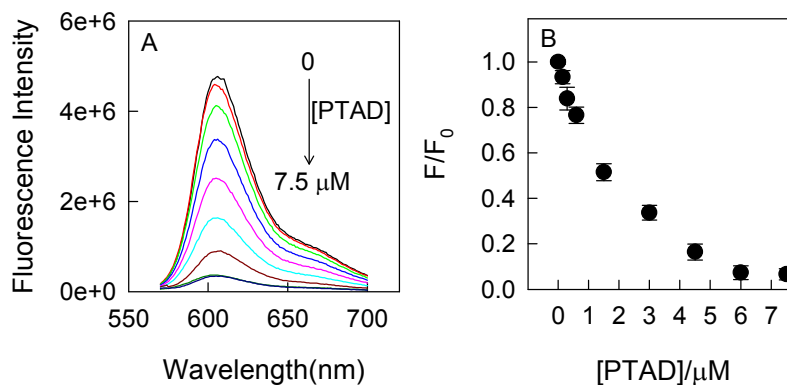


Figure S2. (A) Fluorescence emission spectra ($\lambda_{\text{ex}}=560$ nm) of F_{DNA} (0.25 μM) upon incubation with various amounts of PTAD (0, 0.15, 0.3, 0.6, 1.5, 3.0, 4.5, 6.0, 7.5 μM) in Tris-HCl (20 mM, pH 7.2) at room temperature. (B) The corresponding fluorescence changes upon addition of PTAD in Tris-HCl buffer (20mM, pH=7.2). F_0 and F are the fluorescence intensities of F_{DNA} at 610 nm ($\lambda_{\text{ex}}=560$ nm) before and after treatment with PTAD. Error bars indicate s.d. (n = 3).

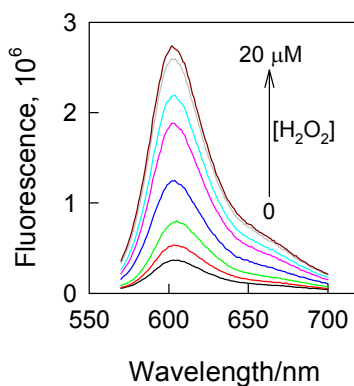


Figure S3. Fluorescence emission spectra ($\lambda_{\text{ex}}=560$ nm) of $F_{\text{DNA}}/\text{PTAD}$ upon incubation with Fe^{2+} (20 μM) and various amounts of H_2O_2 (0, 0.50, 1.0, 2.0, 5.0, 10, 15, 20 μM) in Tris-HCl (20 mM, pH 7.2) at 25 °C.

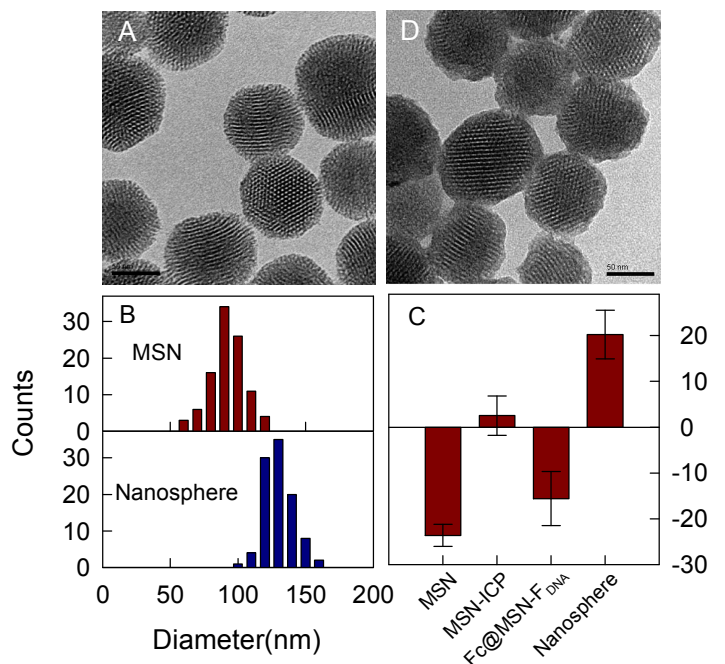


Figure S4. TEM images of the as-synthesized MSN (A) and the nanosphere (D); (B) DLS data of MSN and the nanosphere; (C) Zeta potential of MSN, MSN-ICP, MSN- F_{DNA} , and the nanosphere in Tris-HCl buffer solution (20 mM, pH 7.2).

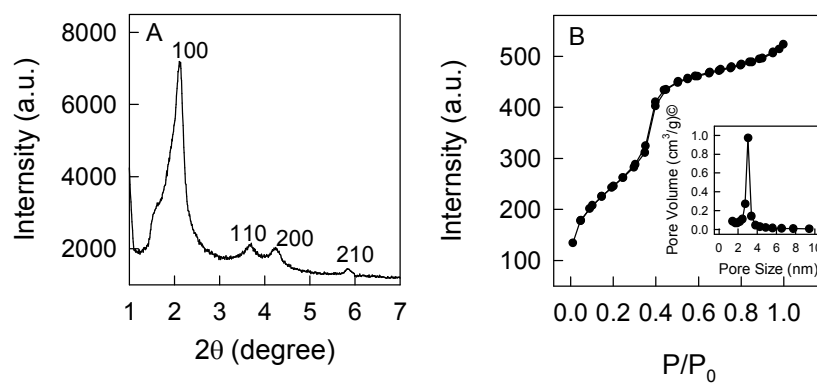


Figure S5. (A) Low angle powder X-ray diffraction patterns (XRD) and (B) Nitrogen adsorption-desorption isotherms of MSN. Insert: pore size distribution curves of MSN.

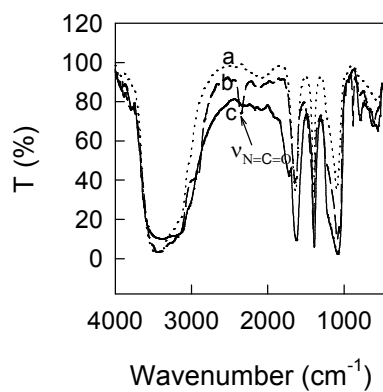


Figure S6. FT-IR spectra of the as-synthesized MSN (a), MSN-ICP (b), and MSN-F_{DNA} (c)

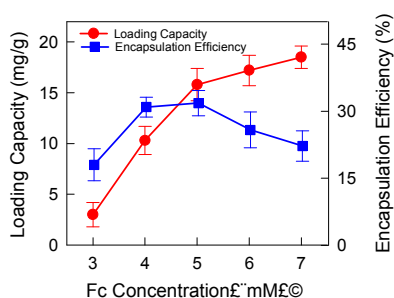


Figure S7. Loading capacity (LC) and encapsulation efficiency (EE) for ferrocene loaded in MSN at different concentrations of stock solutions. The amount of loaded ferrocene was determined by potentiometric titration. With increasing initial weight of ferrocene, the LC is continuously increased, while the EE increases firstly and decreases. Encouragingly, a high LC of 15.8 mg/g SiO₂ and a maximum EE of near 31.8% can be achieved for MSN at 30 mg mL⁻¹.

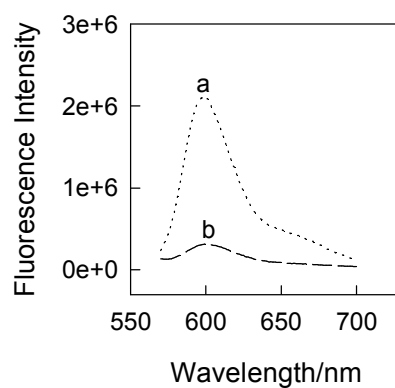


Figure S8. Fluorescence emission spectra of Fc@MSN-F_{DNA} (λ_{ex} = 560 nm) before (a) and after (b) treatment with PTAD in Tris-HCl (20 mM, pH 7.2).

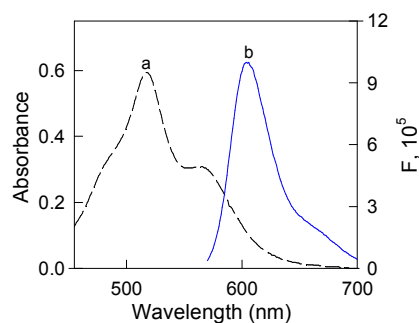


Figure S9. UV-visible absorption spectrum of PTAD (trace a) and fluorescence emission spectra of Fc@MSN-F_{DNA} (trace b, λ_{ex} = 560 nm) in Tris-HCl (20 mM, pH 7.2).

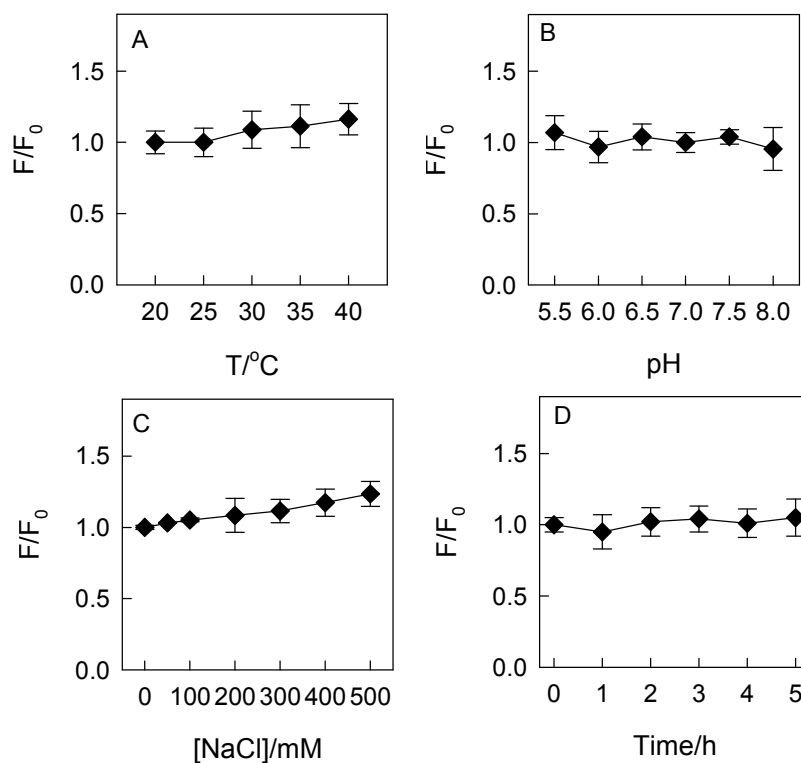


Figure S10. The stability of the nanosphere. Fluorescence intensity of the nanosphere (0.1 mg/mL) at biological temperature (A), biological pH range (B) and various ionic concentration (C). (D) Fluorescence intensity of the nanosphere (0.1 mg/mL) in cell culture media containing 10% (v/v) fetal bovine serum for different time at 37 $^\circ\text{C}$. Error bars indicate s.d. (n = 3).

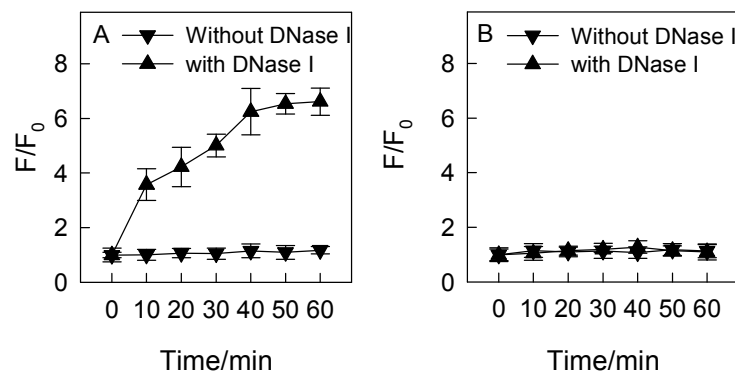


Figure S11. The protective effect of the nanosphere on DNA against nuclease-mediated degradation. Fluorescence emission spectra ($\lambda_{ex}=560$ nm, $\lambda_{em}=610$ nm) of $F_{DNA}/PTAD$ (A) and the nanosphere (B) upon incubation with 3 U/mL DNase I in Tris-HCl (20 mM, pH 7.2) for different time, Where F_0 is the fluorescence intensity at the start of the degradation reaction, F is that at the corresponding time point.

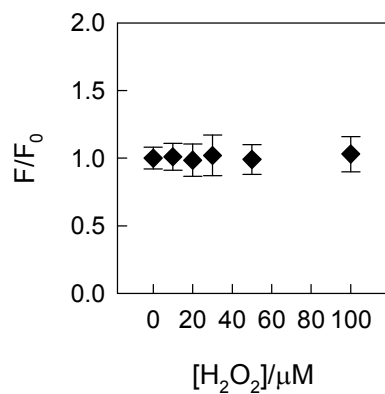


Figure S12. Fluorescence intensity of MSN- $F_{DNA}/PTAD$ in the presence of H_2O_2 at different concentrations. Data were collected after treatment with H_2O_2 for 0.5 h. Error bars indicate s.d. (n = 3).

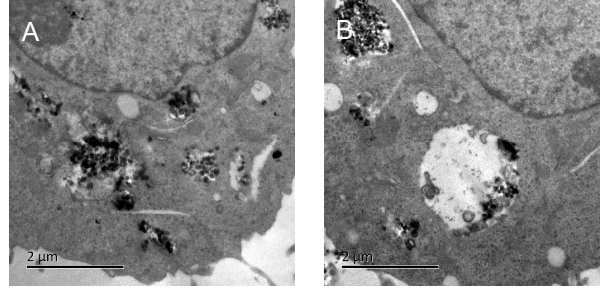


Figure S13. TEM micrographs of the nanosphere (black dot) endocytosed by HeLa cells (A) and HEK 293 cells (B).

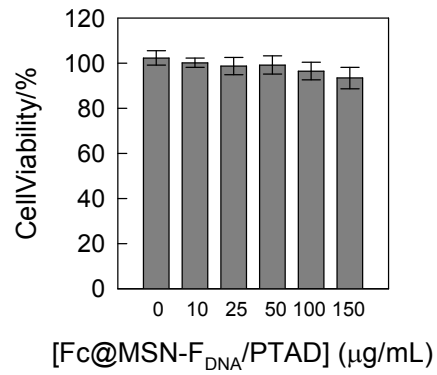


Figure S14. Cell viability of HeLa cells incubated with different concentrations of the nanosphere for 24 h. Cells without treatment by nanosphere were taken as the control, and the viability was set as 100%. Error bars indicate s.d. ($n = 3$).