# Electronic Supplementary Information (ESI)

## A platinum anticancer theranostic agent with magnetic targeting potential derived from maghemite nanoparticles

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### Supplementary Figures

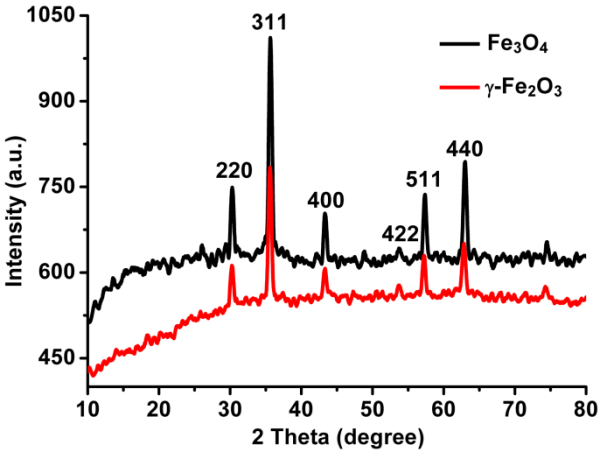



**Fig. S1** X-ray diffraction patterns of $\gamma$-$Fe_2O_3$ and $Fe_3O_4$ NPs. Both kinds of NPs have a highly crystalline nature, with the diffraction peaks matching well with those of the cubic-phase magnemite and magnetite recorded in the JCPDF card (No. 01-1111).

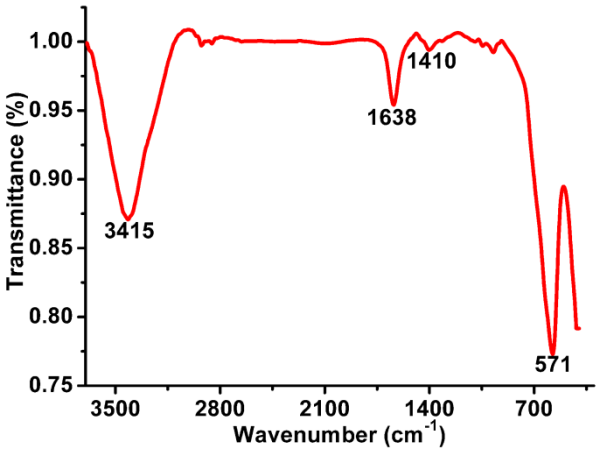



**Fig. S2** FTIR spectra (KBr) of $\gamma$-$Fe_2O_3$ and $Fe_3O_4$ NPs.

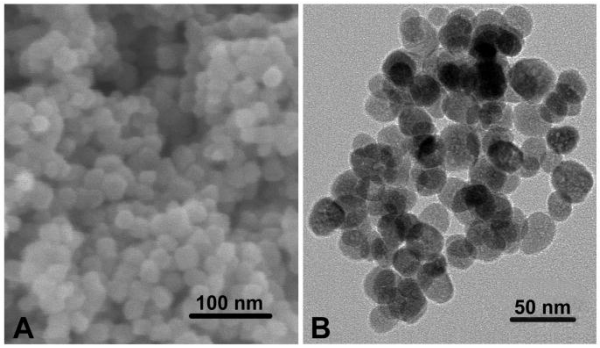



**Fig. S3** SEM (A) and TEM (B) images of $\gamma$-$Fe_2O_3$ NPs.

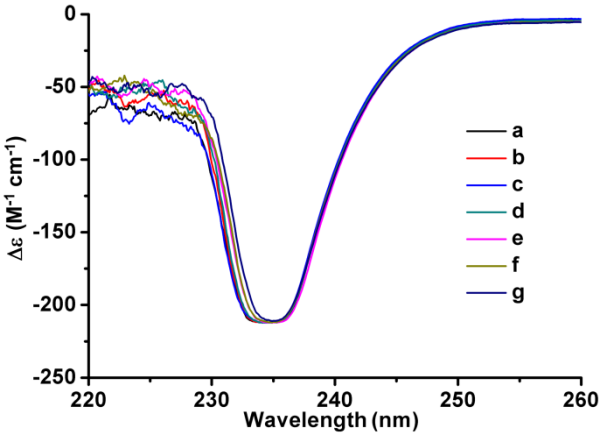



**Fig. S4** CD spectra of fetal bovine serum (10%) in the presence of CMDP-OTPBA-SPION at different concentrations (a, 0; b, 12.5; c, 25.0; d, 50.0; e, 100.0; f, 200.0; g, 300.0 μg $mL^{-1}$) after incubation at 37 °C in PBS buffer (pH 7.4) for 48 h.

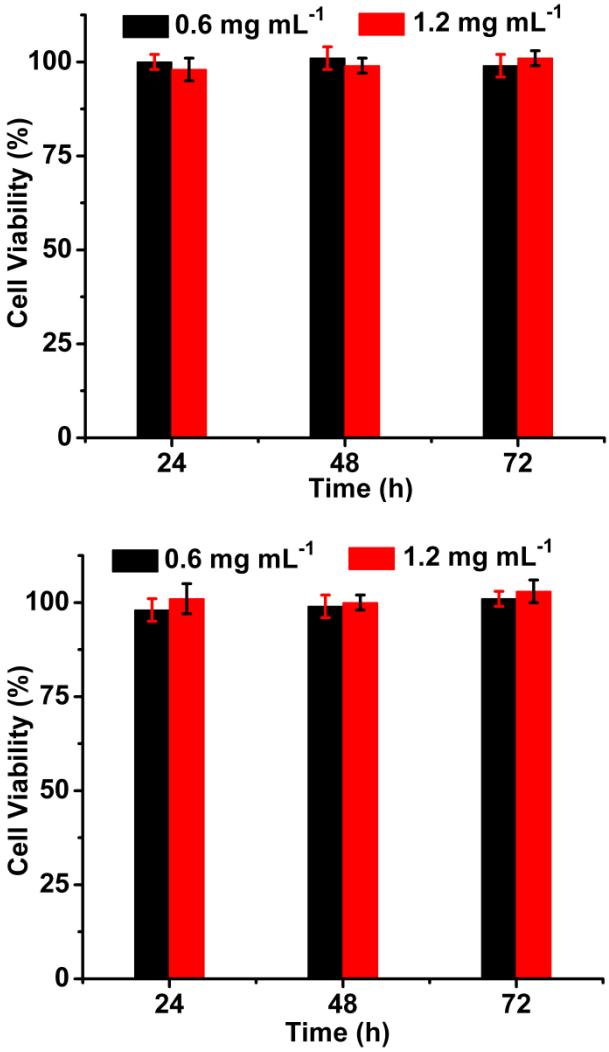



**Fig. S5** Cytotoxicity of OTPBA-SPION against the human cervical cancer cell line HeLa (top) and the human breast adenocarcinoma cell line MCF-7 (bottom) determined by MTT assay at 24, 48 and 72 h, respectively.

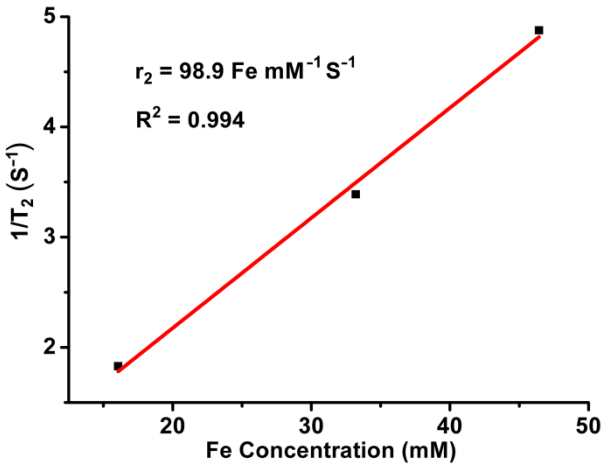



**Fig. S6** $T_2$ relaxation rate ($1/T_2$) versus the Fe concentration in the PBS buffer after CMDP-OTPBA-SPION (150 mg $mL^{-1}$) were incubated with MCF-7 cells in cell culture medium for 3, 6 and 9 h, respectively.

## Experimental procedures

### Materials and methods

$FeCl_3 \cdot 6H_2O$, NaOH, ethylene glycol (EG) and other reagents were of analytical grade and used as received without further purification. 3-Aminopropyltriethoxysilane was purchased from Alfa-aesar. Cisplatin (CDDP) was obtained from Shandong Boyuan Chemical Co., Ltd. Supercoiled pUC19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian). Tris(hydroxymethyl)aminomethane (Tris) and ethidium bromide (EB) were purchased from Sigma. Fetal bovine serum (Cat. No. SH30084.03) was purchased from the Hyclone (U. S. A). Doubly deionized water was prepared on a Milli-Q water system (18.2 MΩ*cm at 25 °C) and used throughout all experiments.

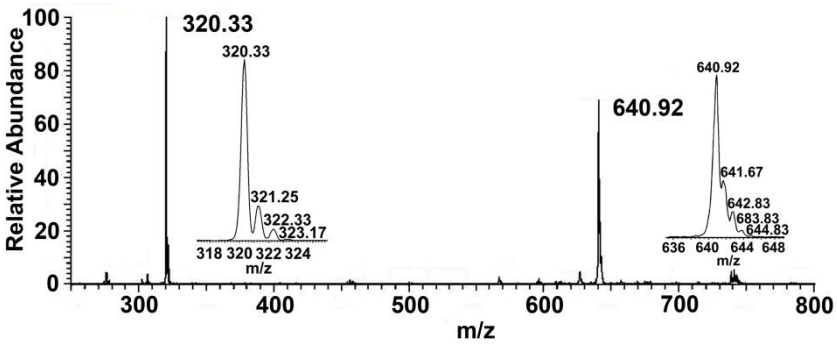
Electrospray mass spectra were recorded using an LCQ electron spray mass spectrometer (ESI-MS, Thermo), and the isotopic distribution patterns were simulated using the ISOPRO 3.0 program. Images of scanning electron microscopy (SEM) were taken using a Hitachi S-4800 field emission electron microscope at an accelerating voltage of 5 kV; and those of transmission electron microscopy (TEM) were obtained using a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. Samples for TEM were prepared by dripping a droplet of dilute phosphate-buffered saline (PBS) sample solution (5 μL) onto a carbon-coated copper grid and drying at room temperature. X-ray powder diffraction (XRD) spectra were recorded on a Japan Shimadzu XRD-6000 diffractometer in the $2\theta$ range of 10−80° with Cu K$\alpha$ radiation ($\lambda$ = 0.15418 nm) and a scanning rate of 0.05 deg $s^{-1}$. X-ray photoelectron spectra (XPS) were acquired on a Thermo ESCALAB 250 electron spectrometer with 150 W monochromatized Al K$\alpha$ radiation (1486.6 eV), where all peaks were referred to the signature C1s peak for adventitious carbon at 284.8 eV. Energy dispersive X-ray spectrum (EDX) was recorded on a Hitachi S-4800 field emission electron microscope at an accelerating voltage of 20 kV. Fourier Transform IR (FTIR) spectra were recorded on a Nicolet 6700 Fourier transform infrared spectrograph in the range of 4000−400 $cm^{-1}$. Field-dependent magnetization were measured on the superconducting quantum interference device (SQUID, Quantum Design, MPMS-XL-7T) magnetometer at 300 K. Zeta potential ($\zeta$) was obtained in water by using a Malven Nano-Z instrument. Hydrodynamic diameters

were determined using a BI-200SM dynamic light scattering system (DLS, Brookhaven Instruments Co., Holtsville, NY). The inductively coupled plasma atom emission spectrometry (ICP-AES) data were determined using a standard Plasma-Quad II instrument (VG Elemental, Thermo Optek Corp.). *In vitro* and *in vivo* MRI were performed on a Siemens Magnetom Trio 3T system.

**Preparation and functionalization of $\gamma$-$Fe_2O_3$ NPs**

$Fe_3O_4$ NPs were first prepared by a modified literature method.[1] In a typical procedure, $FeCl_3 \cdot 6H_2O$ (0.5 g) and NaOH (0.2 g) were sequentially dissolved in EG (15 mL) at 70 °C. The mixture was stirred vigorously to give a clear brown yellow solution, which was transferred into a Teflon-lined autoclave, sealed, kept at 240 °C for 5 h, and then cooled down to room temperature. The black product was collected by magnetic separation, washed with water and ethanol for at least five times to remove the unreacted reagents, and dried in vacuum at room temperature. $Fe_3O_4$ NPs were then oxidized in $HNO_3$ (0.01 M) at 95 °C for 1 h to obtain $\gamma$-$Fe_2O_3$ NPs.

4-Oxo-4-(3-(triethoxysilyl)propylamino)butanoic acid (OTPBA) was synthesized by referring to a literature method.[2] Succinic anhydride (1.0 g) was dissolved in dry dichloromethane (100 mL) at 0 °C under stirring and 3-aminopropyltriethoxysilane (2.21 g) was added slowly into the solution within 1 h. The system was kept reacting at room temperature for 5 h. ESI-MS (m/z) found (calcd): 320.33 (320.16), $[M - H]^-$ ($C_{13}H_{27}NO_6Si$); 640.92 (641.31), $[2M - H]^-$ ($C_{26}H_{53}N_2O_{12}Si_2$).




Finally, OTPBA (0.5 g) was added slowly to the suspension of $\gamma$-$Fe_2O_3$ NPs (30 mg) prepared with isopropanol (200 mL), $H_2O$ (2 mL) and $NH_3 \cdot H_2O$ (1 mL) under ultrasonic wave for 30 min. The mixture was kept at 50 °C for 3 h. The coated MNPs (OTPBA-SPION) were collected by magnetic separation, washed with water and ethanol for at least five times to remove the unreacted OTPBA, and dried into powder at room temperature in vacuum.

**Preparation and characterization of CMDP-OTPBA-SPION**

CMDP was acquired as described previously.[3] OTPBA-SPION was dispersed in the solution of CMDP under sonication and the suspension was stirred vigorously for 48 h. The obtained CMDP-OTPBA-SPION was collected by magnetic separation, and washed with DMF and ethanol under sonication to remove the redundant CMDP. Exhaustive separation was confirmed by examining the Pt content in the supernatant with ICP-AES. CMDP-OTPBA-SPION was characterized by TEM, XPS, EDX, SQUID, and zeta potential. The Pt-loading capacity of OTPBA-SPION was determined by ICP-AES. The sample for ICP-AES was treated with concentrated $HNO_3$ at 95 °C for 2 h. The average of three replicates was taken as the final result.

The transverse relaxation time ($T_2$) of CMDP-OTPBA-SPION was measured at ascending concentrations of Fe in the PBS buffer on a 3.0 T MR scanner (Magnetom Avanto, Siemens, Germany) with a spin-echo pulse sequence under the following conditions: matrix size = 122 × 256, field of view

(FOV) = 100 mm × 170 mm, slice thickness (SL) = 3 mm, echo time (TE) = 11.7 ms, repetition time (TR) = 1500 ms, number of acquisitions = 1. The transverse relaxivity ($r_2$) was calculated according to the following equation:[4]

$$1/T_2 = 1/T_2^0 + r_2 \cdot [Fe]$$

where $1/T_2$ is the relaxation rate in the presence of CMDP-OTPBA-SPION, $1/T_2^0$ is the relaxation rate of pure water, and [Fe] is the concentration of CMDP-OTPBA-SPION in terms of Fe.

**Stability of the nanocomposite**

CMDP-OTPBA-SPION (2.0 mg) was dispersed in water (pH 7.0, 10 mL), PBS (pH 7.4, 10 mL) and HAc-NaAc (pH 5.2, 10 mL) under ultrasonication respectively. The suspension was transferred into a dialysis bag and dialyzed in water (16 mL) for 24, 48 and 72 h respectively at 37 °C. The Pt content in the outside solution was determined by ICP-MS.

**Agarose gel electrophoresis and cellular uptake**

Supercoiled pUC19 DNA (20 ng $\mu L^{-1}$) was treated with ascending concentrations of CMDP-OTPBA-SPION in the buffer of pH 7.4 (50 mM Tris-HCl, 50 mM NaCl) and pH 5.2 (50 mM HAc-NaAc, 50 mM NaCl), respectively. The mixtures (10 μL) were incubated at 37 °C for 24 h. The resulting solutions were loaded onto the agarose gel (1%) and subjected to electrophoresis in a TAE buffer (40 mM Tris acetate, 1 mM EDTA). DNA bands were stained by EB, visualized under UV light and photographed.

MCF-7 cells ($2.5 \times 10^5$) were seeded in plates containing Dulbecco's modified Eagle's medium and incubated at 37 °C in the humidified atmosphere with 5% $CO_2$ for 24 h. The cells were then treated with fresh medium containing CMDP-OTPBA-SPION (15 μg $mL^{-1}$, in terms of Pt) and blank control, respectively, and incubated at 37 °C for 24 h. The plates were washed with PBS (4 mL) thrice to remove the dead cells and remnant composite. The cells were digested by pancreatic enzyme and washed with PBS (2 mL) five times to remove the composite outside the cells. The Tyndall effect of the cells was recorded in the PBS solution in the cuvette using a laser pointer and a camera in the absence or presence of an external magnetic field.

**Cytotoxicity assay**

The cytotoxicity of CMDP-OTPBA-SPION was tested by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with CDDP and OTPBA-SPION as the references. Briefly, HeLa and MCF-7 cancer cells were seeded respectively in 96-well plates at 5000 cells per well in Dulbecco's modified Eagle's medium and incubated at 37 °C in the humidified atmosphere with 5% $CO_2$ for 16 h. The cells were then treated in triplicate with fresh medium containing grade concentrations (in terms of Pt) of CMDP-OTPBA-SPION, CDDP, and OTPBA-SPION, respectively. Aliquot of MTT (20 μL, 5 mg $mL^{-1}$) PBS solution was added to each of the wells after the cells were incubated at 37 °C for 24, 48 and 72 h, respectively. The supernatant was removed after 4 h of incubation and DMSO (200 mL) was added to each well. The remanent composite was attracted to the bottom of the plate by a magnet for 20 min, and the supernatant (100 mL) containing the resultant MTT formazan was determined at 570 nm using an ELISA plate reader. The cytotoxicity was calculated based on the data of four replicate tests.

**In vitro and in vivo MRI**

MCF-7 cells ($5 \times 10^5$) were incubated with CMDP-OTPBA-SPION (150 μg $mL^{-1}$) for 3, 6, and 9 h at 37 °C, respectively, in cell culture medium, and then were washed with PBS (4 mL) and cell culture medium five times and suspended in PBS (1 mL, $2 \times 10^5$) for MRI. After that, the cells were digested in ultrapure concentrated $HNO_3$ at 95 °C for 8 h and diluted to 2 mL with $H_2O$ to determine the Fe content using a high-resolution sector field ICP-AES (VARIAN Technologies China). Data were acquired at medium resolution (4000) using Fe (5 ppb) as an internal standard. $T_2$-weighted images were acquired using spin-echo imaging sequence with the following parameters: matrix size = 83 × 256, FOV = 65 mm × 160 mm, SL = 3 mm, TE = 11.7 ms, TR = 1500 ms, number of acquisitions = 1.

*In vivo* assays were carried out according to the protocol approved by the Institutional Animal Care and Use Committee. RM1 murine prostate cancer cells ($5 \times 10^5$) were implanted subcutaneously in 9-week old male C57BL/6J mice with body weight of approximately 30 g to form palpable tumors in 20 to 25 days. Endpoint assays were conducted when the tumors were visible and reached 15 mm × 15 mm in size. Mice were anaesthetized with trichloroacetaldehyde hydrate (10%) by intraperitoneal injection. CMDP-OTPBA-SPION (5.0 mg $kg^{-1}$ of body weight) was injected intravenously. External magnetic field was exerted on the mouse by fixing a circle magnet (diameter = 15 mm) to the tumor area. MRI studies were performed at 0 and 2 h after the injection, respectively. $T_2$-weighted images were acquired using spin-echo imaging sequence with the following parameters: matrix size = 512 × 512, FOV = 50 mm × 50 mm, SL = 0.8 mm, TE = 41 ms, TR = 4500 ms, number of acquisitions = 1.

## References

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