# Electronic Supplementary Information

## Cucurbit[7]uril-functionalized Magnetic Nanoparticles for

## **Imaging-guided Cancer Therapy**

Ludan Yue, Chen Sun, Cheryl H.T. Kwong, and Ruibing Wang\* State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China

#### 1. Experimental Section

#### Materials and Characterization

Ferric acetylacetonate (Fe(acac)<sub>3</sub>), ethanol, paraformaldehyde, 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT), folic acid, amantadine,1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and N-hydroxy succinimide (NHS) were purchased from Aladin and used as received. Oleic acid and oleic amine were purchased from TCI (tcichemicals, Shanghai, China). Oxaliplatin was purchased from Yuanye biological company (China).

Milli-Q Integral system (Merck) was used to supply Milli-Q water in this work. The morphology of NPs was observed by transmission electron microscopy (TEM, JEOL 2100F, Japan) at 200KV. The Zeta potential of nanomaterials was measured on a Zetasizer (Malvern). NMR spectra were obtained using a Bruker Ultra Shield 600 PLUS NMR spectrometer. Fluorescence spectra were obtained on fluorescence spectrometer (Thermo Scientific Lumina). Fluorescence images were acquired by confocal laser scanning microscopy (CLSM, Leica TCS SP8, German) and inverted fluorescence microscope (Olympus IX73). Fourier transform infrared (FT-IR) spectra and UV-vis-NIR absorbance spectra were measured on IFS-66V/S and Shimadzu UV-1800 spectrophotometers. MTT assays were measured using a microplate reader (Infinite F200 Pro, TECAN). Intracellular uptake and cell apoptosis were both quantified by a flow cytometer (Beckman coulter).

#### Preparation of Fe<sub>3</sub>O<sub>4</sub> NPs

Fe<sub>3</sub>O<sub>4</sub> NPs were synthesized following a previously reported method<sup>1</sup>. Briefly, 2 mmol Fe(acac)<sub>3</sub>, 10 mmol 1,2-hexadecanediol, 6 mmol oleic acid and 6 mmol oleic amine were dissolved in benzylether under argon. The mixture was stirred and heated to 300 °C at the rate of 4 °C/min followed by refluxing for another 30 min. After cooling to room temperature, the obtained black magnetic nanoparticles were washed by excessive ethanol.

#### Preparation of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs

2 mL DMSO with 20 mg DMSA was added to 2 mL toluene containing 20 mg of the  $Fe_3O_4$  NPs and stirred for 12 hours. The obtained precipitant was then washed with ethyl acetate and finally stored in 2 mL Milli-Q.

AO<sub>1</sub>CB[7] was synthesized following Kim's method<sup>2, 3</sup> and confirmed by NMR. The AO<sub>1</sub>CB[7] was conjugated with Fe<sub>3</sub>O<sub>4</sub> NPs via a "click" reaction between AO<sub>1</sub>CB[7] and DMSA under UV irradiation. The excessive AO<sub>1</sub>CB[7] was washed by Milli-Q.

The amount of CB[7] on the surface was calculated by a fluorescence assay. Briefly, an excessive amount of the fluorescent guest molecular AO was used to bind with the CB[7] to form CB[7]·AO. A known quantity of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was mixed with an excessive amount (amount A) of ADA in water and shake for 5 min before ultra-centrifugation. The ADA (amount B) remain in the supernatants was measured by adding CB[7]·AO and the fluorescence was corresponded in the CB[7]·AO standard curve(y=-2049.1+2529, Fig. S3). A minus B is the amount of CB[7].

#### TGA of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs

20 mg CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was used for TGA under nitrogen protection. The weight loss was recorded from room temperature to 700 °C. Fe<sub>3</sub>O<sub>4</sub> NPs, AO<sub>1</sub>CB[7] and the mixture of CB[7] and Fe<sub>3</sub>O<sub>4</sub> NPs were analyzed as the control.

#### Preparation of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs

CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs were surface-functionalized by simple mixing the NPs with functional tags, such as FA-Ada, FITC-Ada and OX, and subsequent centrifugation to remove excess ligands. The drug loading content was measured according to Pt content of OX by ICP-MS.

FITC/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was produced with the same method for studying the cellular uptake **Release profile of OX in Vitro** 

The OX release profile of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was assessed in vitro by using a dialysis method. Phosphate-buffered saline (PBS) solution containing 0, 0.1 and 1 mM concentrations of spermine (at room temperature) was used as the release medium to study the OX release kinetics. 100  $\mu$ L dialysate was suck out and replenished at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 5, 10, 20, 30, 48, 72h. The OX release was quantified by ICP-MS.

#### Cell Culture and in Vitro Cytotoxicity Assays.

The 4T1 cell line (mice breast cancer cell line) and L02 cell line (human fetal hepatocyte line) were cultured in 25 cm<sup>2</sup> flasks using Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin & streptomycin (PS) solution at 37 °C with 5% CO<sub>2</sub>.

The biocompatibility of NPs was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays using 4T1 and L02 cell lines ( $1 \times 10^4$  cells per well). The cellular viability was calculated following a previously published procedure.

#### Cellular uptake

The MCF-7, 4T1 and L02 cells were incubated by NPs and free FITC at an equal concentration of FITC for 12 h, respectively.

The internalized fluorescence images were acquired by a confocal laser scanning microscope (CLSM) on the cells after washing with PBS. The cellular fluorescence signal was obtained by flow cytometry assay.

#### Cellular Apoptosis Evaluation.

The apoptosis rate of MCF-7, 4T1 and L02 cells after incubation with NPs was evaluated by flow cytometry assay. Briefly, the cells after incubation were processed by an Annexin/V-FITC Kit. The fluorescence signal of annexin V-FITC and PI obtained by flow cytometer reflected the apoptosis rate.

#### **Biosafety and Cytotoxicity**

The biosafety of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was studied via MTT assays. Briefly, the MCF-7, 4T1 and L02 cell line were seeded in 96-well plates with  $10^4$  cells/well density in 100 µL of DMEM (with 10% FBS and 1% PS), respectively, and subsequently incubated with CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs (50, 100, 150, 200, 250 µg/mL Fe). The absorbance value was measured by a microplate reader.

The cytotoxicity of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs was studied via MTT assays. Briefly, the MCF-7, 4T1 and L02 cell line were seeded in 96-well plates with  $10^4$  cells/well density in 100 µL of DMEM (with 10% FBS and 1% PS), respectively, and subsequently incubated with OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs and free OX (at Fe concentration of 0.5, 2,5, 5, 25, 50 µg/mL and Pt concentration of 0.342, 1.71, 3.42, 17.12, 34.23 µM) for 24 and 48 h, respectively. The absorbance value was measured by a microplate reader.

#### In Vivo Antitumor Study.

In vivo antitumor effect of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs were studied by 4T1 tumor-bearing balb/c mice. 12 balb/c mice were subcutaneously injected with  $10^6$  4T1 cells( $10^7$  cells per mL PBS solution ). When the average volume of the tumor reached about 50 mm<sup>3</sup>, the mice were random grouped into 4 groups according to the disposal condition: PBS, free OX, FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs, OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs.( at Fe concentration of  $100 \mu$ g/mL and Pt concentration of 68.45  $\mu$ M for 200  $\mu$ L). The PBS and all NPs were injected intravenously into the tumor-bearing mice on day 0. Tumor size and bodyweight of mice were measured and recorded every 2 days.

#### Histological Staining.

On the 14th day after administration, the mice were sacrificed and their major tissues, including heart, liver, spleen, lung, and kidney, were excised to perform the histological assay.

The organs and tumors were fixed in 4% paraformaldehyde and the tissue section stained with hematoxylin–eosin (H&E) was observed under an IX73 microscope (OLYMPUS, Japan). All animals used in the procedures were handled in strict accordance with the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the University Committee in the Use of Animals of University of Macau.

#### 2. Supplementary Results



Fig. S1. Materials Characterization. (A) TEM image and size distribution of Fe<sub>3</sub>O<sub>4</sub>-DMSA NPs (Scale bar: 20 nm). (B) Zeta potentials of Fe<sub>3</sub>O<sub>4</sub>-DMSA NPs, and CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs. (C) DLS analysis of Fe<sub>3</sub>O<sub>4</sub>-DMSA NPs and CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs.



Fig. S2. The <sup>1</sup>H NMR spectrum of  $AO_1CB[7]$  in  $D_2O$ .



Fig. S3. Fluorescence intensity ( $\lambda_{ex} = 450 \text{ nm}$ ) and standard curve of CB[7]·AO with different amount of ADA. (2 mM AO, 1.6 mM CB[7]).



Fig. S4. Materials Characterization. (A) TEM image and size distribution of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs (Scale bar: 20 nm). (B) Zeta potential of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs. (C) DLS analysis of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs.



Fig. S5. MTT assays of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs with MCF-7, 4T1 and L02 cell lines upon incubation of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs at Fe concentration of 50, 100, 150, 200, 250  $\mu$ g/mL for 24 and 48 h, respectively.



Annexin-FITC

Fig. S6. Flow cytometry assay of MCF-7, 4T1 and L02 cells after 24 h incubation of CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs at Fe concentration of 250  $\mu$ g/mL.



Fig. S7. Fluorescence images of MCF-7 and 4T1 cells with (or without) 4 h pre-incubation of excessive FA before 24 h incubation of FITC/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub>NPs.



### Annexin-FITC

Fig. S8. The apoptosis rate of MCF-7 cells(A and D), 4T1 cells(B and E) and L02 cells(C and F) after 24 incubation of OX/FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs and OX at Fe concentration of 50  $\mu$ g/mL and Pt concentration of 34.23  $\mu$ M.



Fig. S9. The corresponding  $1/T_2$  value of tumor site of 4T1 tumor bearing mice post intravenous injection of FA/CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs and CB[7]-Fe<sub>3</sub>O<sub>4</sub> NPs (at 1 mg/mL Fe concentration) for 0, 2, 6 and 12 h, respectively.

#### Reference

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