

## Supporting Information

### **Inherent anchorage in UiO-66 nanoparticles for efficient capture of alendronate and its mediated release**

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## **1. Experimental details**

### **1.1 Synthesis of UiO-66 Nanoparticles**

ZrCl<sub>4</sub> (466 mg, 2 mmol), terephthalic acid (BDC, 320 mg, 2 mmol), benzoic acid (2.44 g, 20 mmol) and HCl (37%, 12 M; 0.33 mL, 4 mmol) in 36 mL of DMF were ultrasonically dissolved in a Pyrex vial. The mixture was heated in an oven at 120 °C for 48 h. After cooling down to room temperature, a white powder of UiO-66 nanoparticles (NPs) was harvested by centrifugation and washed with DMF at room temperature.

### **1.2 Sample Activation**

Before the drug loading experiment, the as-synthesized UiO-66 NPs were washed with DMF and dispersed in DMF at room temperature under stirring for 6 h in order to remove the free BDC. Finally, the same procedure was repeated twice using acetone instead of DMF to exchange the trapped DMF. The mixture was finally centrifuged, and the acetone was removed by decanting. The obtained NPs were dried at 80 °C in vacuum oven overnight.

### **1.3 Synthesis of UiO-66-FMN**

To prepare the UiO-66 labeled with fluorescent agent for microscopy observation, Flavin mononucleotide (FMN, 3 mg) and activated UiO-66 (20 mg) were dissolved in 8 mL water at room temperature under vigorous stirring for 24 h in dark. The resultant FMN grafted UiO-66 (UiO-66-FMN) were collected by centrifugation at 10000 rpm for 15 min, and the supernatant was then taken out for fluorescence measurement. The decrease of the fluorescence intensity in the supernatant corresponds to the adsorption amount of FMN onto UiO-66. The product was washed with water several times to remove the physically adsorbed FMN. Finally UiO-66-FMN was dried at 60 °C in vacuum oven for 24 h.

### **1.4 Characterization and Measurements**

Powder X-ray diffraction (XRD) patterns were collected on Bruker D8 equipped with Cu K $\alpha$  radiation (40 kV, 40 mA) at a rate of 6 ° min<sup>-1</sup> over the range of 4-40 ° (2 $\theta$ ). FTIR spectra were recorded on a Nicolet 7000-C spectroscopy with a resolution of 4 cm<sup>-1</sup> using the KBr method. N<sub>2</sub> adsorption-desorption isotherms were obtained with a Quantachrome NOVA 4200E porosimeter at -196 °C under a continuous adsorption condition. All samples were degassed in a vacuum at 100 °C for 12 h before measurements. The surface area and micropore volume were calculated by the Brunauer-Emmett-Teller (BET) using adsorption data at a relative pressure lower than 0.15. UV-visible absorption spectra were determined with a Shimadzu UV-vis 3101 spectroscopy. Field Emission Scanning Electron Microscopy (FE-SEM) was performed on FEI Magellan 400 electron microscope. TEM images were taken with a JEOL JEM-2100 microscope operating at 200 kV.

### 1.5 Loading Anti-cancer Drug of Alendronate

Several batches of UiO-66 NPs (160 mg) were immersed in 100 mL of 2 mg mL<sup>-1</sup> alendronate (AL) solution at pH 4.8 (HCl aqueous solution) at room temperature under vigorous stirring. After stirring for 12 h at 37 °C, the resulting suspension was centrifuged at 10000 rpm for 20 min. The supernatant were collected and the residual AL content was measured by ICP-AES to determine the AL loading amount.

### 1.6 *In vitro* AL Release

To measure *in vitro* drug release, **two batches of 50 mg UiO-66-AL** were suspended in 5 mL of PBS (5 mM) with pHs of 5.5 and 7.4. The suspensions were placed into pretreated dialysis bags with a molecular weight cut off of 3000 Da and sealed with dialysis bag holders. The sealed dialysis bag was put into a beaker with 50 mL of PBS with the same pH conditions. The beaker was shaken at 100 rpm at 37 °C. At certain time intervals, 0.2 mL of the release medium was taken out. The volume of the dissolution media was maintained at 50 mL. The AL concentration was measured by spectrophotometric through their oxidation using excess ceric (IV) sulphate in the presence of 0.5 M sulphuric acid at room temperature. The amount of the consumed ceric equivalent to the concentration of AL was calculated by

measuring the absorbance of ceric solution (as blank) against the test solution at 320 nm.<sup>1</sup> These measurements gave the information on the quantity of released AL.

### **1.7 *In vitro* Biocompatibility of UiO-66 NPs**

For the biocompatibility studies of UiO-66, MCF-7 and HepG2 cells were seeded in a 96-well plate at a density of  $10^4$  cells per well and were incubated in DMEM media containing 10% fetal calf serum for 24 h at 37 °C. Then, the above activated UiO-66 NPs was added to the media, the concentration of UiO-66 was set as 25, 50, 100, 200 and 300  $\mu\text{g mL}^{-1}$ , respectively. At the end of each incubation (24 h, 48 h), 10  $\mu\text{L}$  of 5  $\text{mg mL}^{-1}$  MTT solution in PBS (pH 7.4) was added to each well. After the cells were incubated for another 4 h, the medium was withdrawn and 100  $\mu\text{L}$  DMSO was added to each well to dissolve the MTT formazan crystals. Then, the absorbance of each well was measured at 490 nm in a microplate reader. Cell viability was calculated as a percentage of viable cells after treated with nanoparticles compared with the untreated cells.

### **1.8 Cellular Uptake of UiO-66 Nanocarriers Monitored with LSCM**

For Laser Scanning Confocal Microscope (LSCM) observations,  $2 \times 10^4$  HepG2 cells were seeded in a LSCM-special cell culture dish and incubated in DMEM media containing 10% fetal calf serum for 12 h at 37 °C. UiO-66-FMN were dispersed into DMEM solutions with a concentration of 100  $\mu\text{g mL}^{-1}$  and then added into the culture dish. After co-incubation for 4 h, the cells were washed three times with PBS (pH 7.4) to remove the residual UiO-66-FMN. Cells were fixed by formaldehyde (4%) for 20 min at 37 °C and stained by DAPI, and then washed three times by PBS (pH 7.4). Finally, the confocal dishes were visualized under a laser scanning confocal microscope (FluoView FV1000, Olympus).

### **1.9 Flow Cytometry Assay**

To evaluate the cellular uptake of nanocarriers, cell culture dish containing  $2 \times 10^5$  HepG2 was incubated with UiO-66-FMN (1 mL, 100  $\mu\text{g mL}^{-1}$ ) in DMEM media containing 10% fetal calf serum at 37 °C for 4 h and 12 h. The cells incubated with blank UiO-66 were used as controls. After being washed twice with PBS to remove the unbound nanoparticles, the cells were harvested with trypsin. The

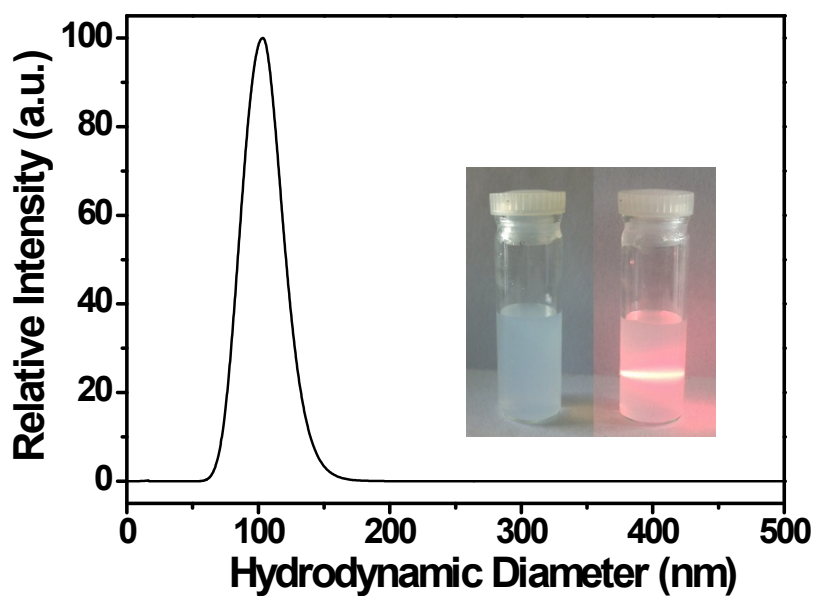
collected cells were resuspended in 1 mL of PBS and analyzed with flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA).

#### **1.10 *In vitro* Cytotoxicity of Free AL and AL-UiO-66**

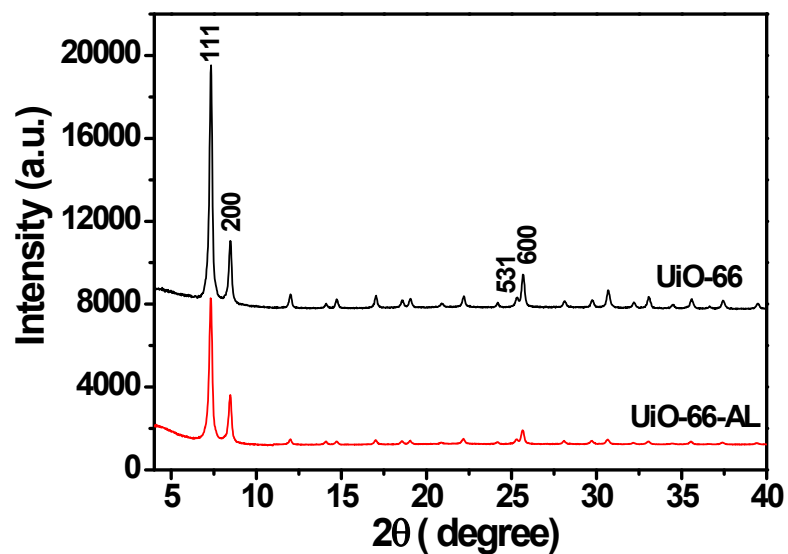
For the cytotoxicity studies of free AL and AL-UiO-66, MCF-7 and HepG2 Cells were seeded in a 96-well plate at a density of  $10^4$  cells per well and were incubated in DMEM media containing 10% fetal calf serum for 24 h at 37 °C. Then free AL and AL-UiO-66 were added to medium and the cells were incubated at 37 °C for 24 and 48 h. The drug concentrations were set as 20, 40, 60, 100  $\mu\text{g mL}^{-1}$  on an AL basis. The nanoparticle concentrations were also varied according to the drug concentrations. Cell viability was determined by the standard MTT assay, which was the same as the procedure for cytotoxicity assay for the UiO-66 nanocarriers.

#### **References**

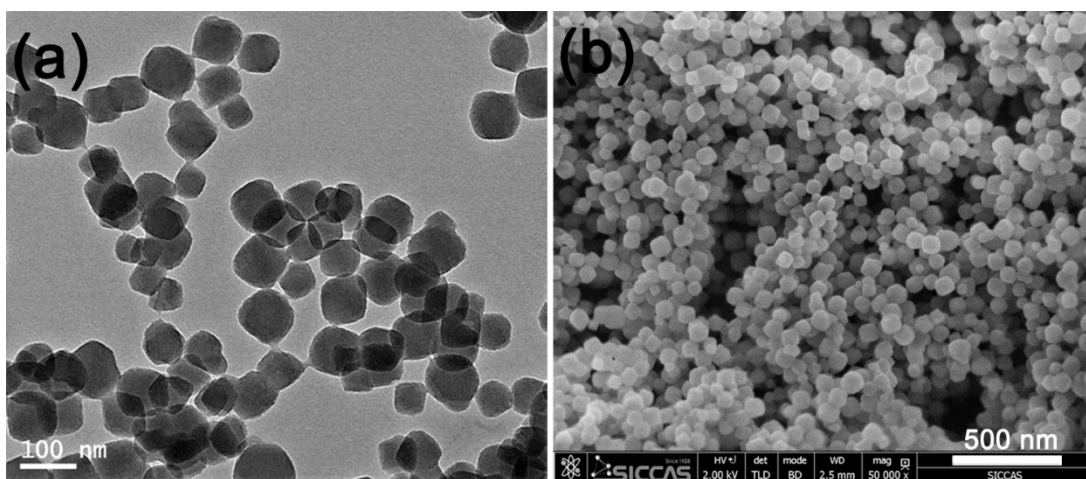
- (1) J. Gu, M. Huang, J. Liu, Y. Li, W. Zhao and J. Shi, *New J. Chem.*, 2012, **36**, 1717.



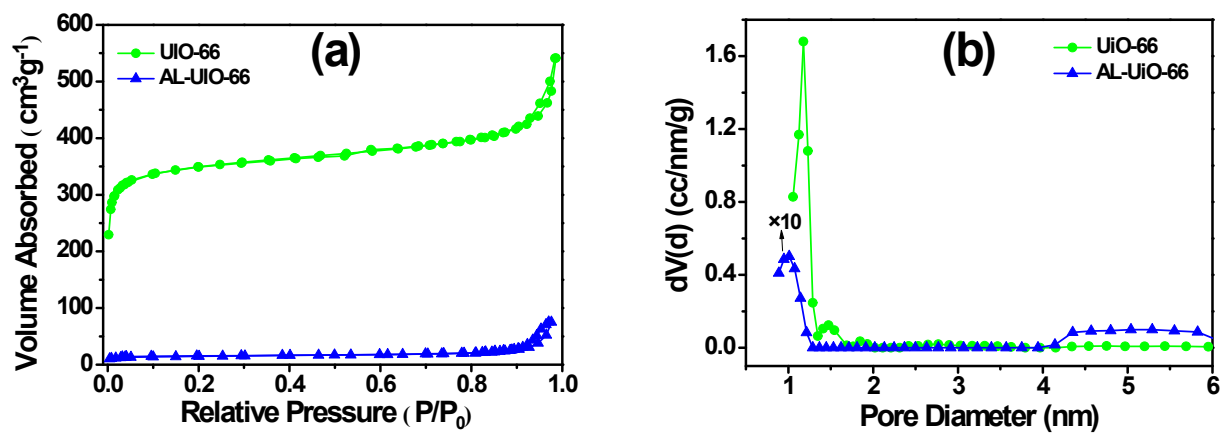
**Fig. S1** Typical DLS profile of UiO-66 NPs measured in aqueous solution and the inset demonstrates the optical photographs of Tyndall phenomenon by illuminating the nanoparticles suspension in water.



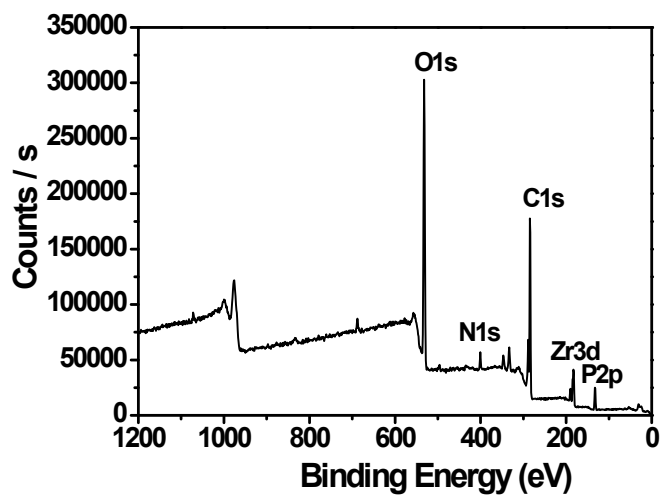
**Fig. S2** XRD patterns for the UiO-66 before (black) and after (red) drug loading.



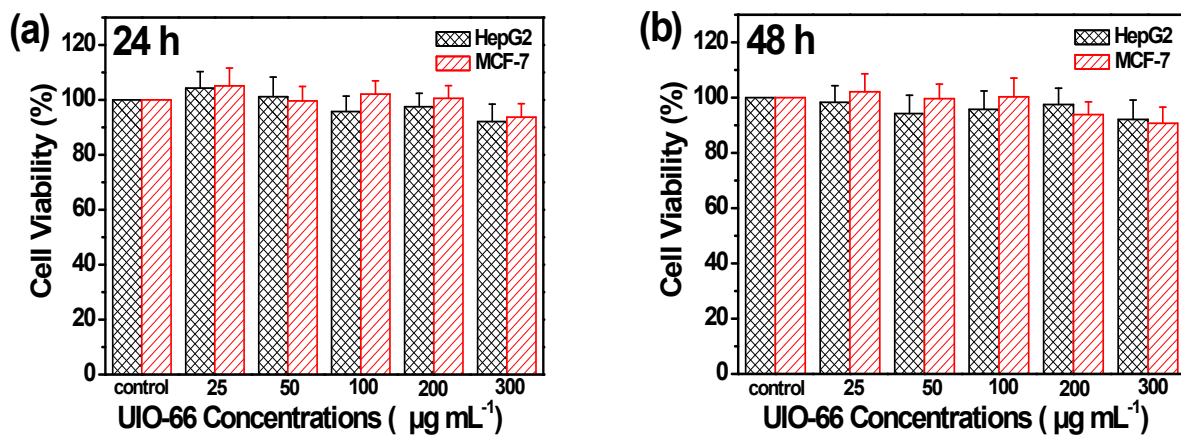
**Fig. S3** (a) TEM and (b) FE-SEM images of the AL-Uio-66.



**Fig. S4** (a) Nitrogen adsorption-desorption isotherms of UiO-66 and AL-Uio-66. (b) The corresponding DFT pore size distribution.

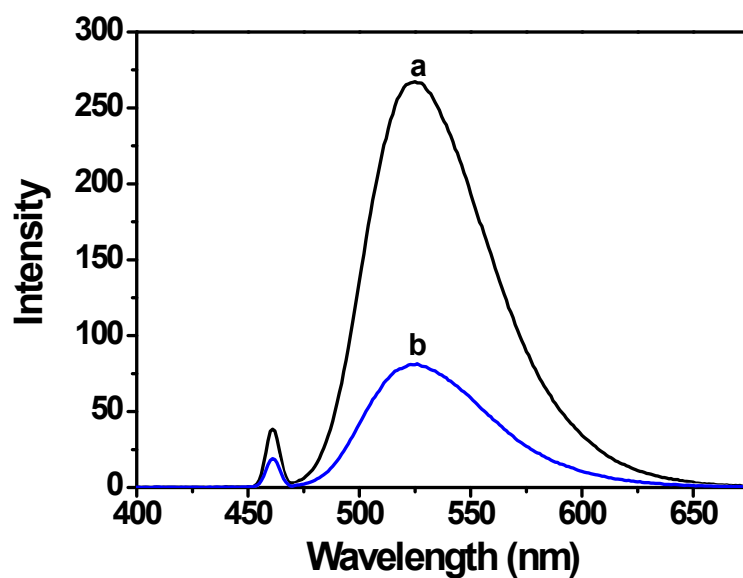


**Fig. S5** Wide scan XPS spectrum of AL-UiO-66.

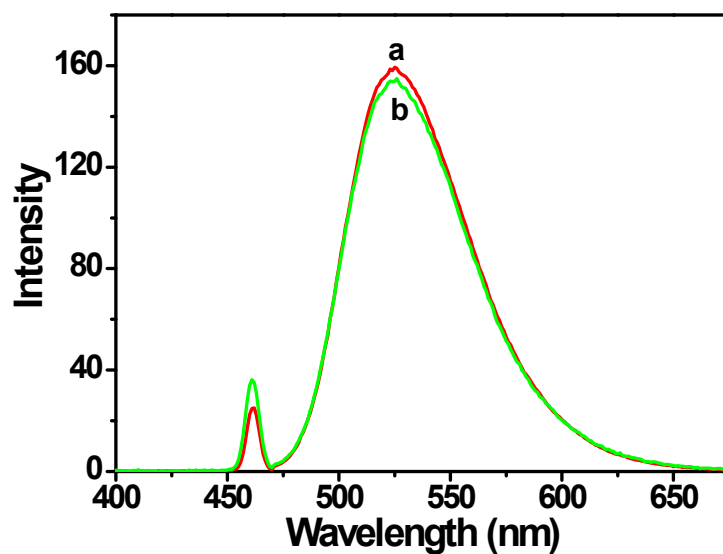


**Fig. S6** Cell viabilities of UiO-66 against HepG2 cells and MCF-7 cells at different concentrations with incubation time of 24 (a) and 48 (b) h.

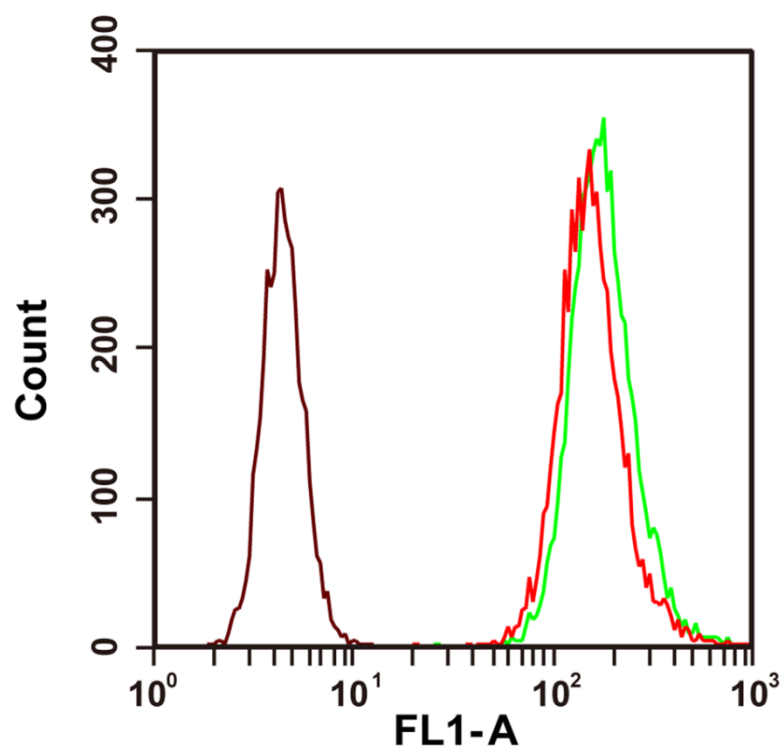




**Fig. S7** Fluorescence emissions of FMN (a) before and (b) after UiO-66 NPs adsorption. The reduced emission from FMN was due to the bonding of fluorescent molecules onto the surface of UiO-66 NPs.



**Fig. S8** Fluorescence spectra of UiO-66-FMN (a) before and (b) after their soaking in water. The change of fluorescence intensity was negligible implied that almost no FMN was leaked from the UiO-66 NPs within 24 h.



**Fig. S9** Flow cytometric analyses of HepG2 cells after incubation with  $100 \mu\text{g mL}^{-1}$  UiO-66-FMN for 4 (red) and 12 h (green) at  $37^\circ\text{C}$ .