Electronic Supplementary Information (ESI)

Facile one-pot synthesis of carbon/calcium phosphate/Fe_3O_4 composite nanoparticles for simultaneous imaging and pH/NIR-responsive drug delivery

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Experimental Section

Chemicals: Polyacrylic acid (PAA, Mw ≈ 1800) and doxorubicin hydrochloride (DOX) were procured from Sigma (USA). Calcium hydroxide, isopropyl alcohol (IPA), iron (Ⅱ) chloride tetrahydrate (98%) and diammonium hydrogen phosphate (99%) were purchased from Sinopharm Chemical Reagent Beijing Co, Ltd. Deionized water (DI water) was used in all experiments.

Characterization: TEM was recorded with a JEOL-2100F

transmission electron microscope. SEM and EDX were carried out with a JEOL JSM-7610F scanning electron microscope. HR-TEM was performed on a TECNAI G2 F20 transmission electron microscope under 200 kV accelerating voltage. XPS were measured on an ECSALAB 250 using monochromatic Al-Kα radiation. The magnetic measurement was obtained by using a superconducting quantum interference device magnetometer (SQUIDMPMS XL-7) with fields up to 1.5 T. FTIR spectroscopy spectra were performed by a Magna 560 FTIR spectrometer. XRD patterns were measured by using a D8 Focus di□ractometer (Bruker) equipped with Cu Kα radiation. UV-vis absorption spectroscopy was carried out by U-3010 spectrophotometer (Hitachi, Japan). Pore size and surface area were analyzed by N_2 adsorption/desorption measurements using an intelligent gravimetric analyser Autosorb-iQ (Quantachrome). TG analysis was carried out on a Perkin-Elmer TG-7 analyzer heated from room temperature to 800 ^{o}C at a ramp rate of 10 ^{o}C min^{-1} in air. T_2 -weighted MRI and the transverse relaxation time T_2 were performed by using a 1.2 T MRI instrument (Shanghai Huantong Corporation HT-MRSI50-50KY).

Synthesis of carbon/CaP/Fe_3O_4 composite NPs: In a 500 mL flask, PAA (1 mL, 0.2 g/mL) and 60 mg of $Ca(OH)_2$ were mixed in 100 mL of DI water with magnetic stirring for 30 min. Then, 200 mL of IPA was dropwise added into the mixture to obtain PAA-Ca nanoparticles. After

that, $FeCl_2$ solution (100 mg of $FeCl_2$ in 0.2 mL DI water) was injected into the mixture. With continuous stirring for 2 h, $(NH_4)_2HPO_4$ solution (71 mg of $(NH_4)_2HPO_4$ in 0.2 mL DI water) was added four times for every 30 min intervals. The reaction was carried out for 12 h with continuous stirring at room temperature to obtain PAA/CaP/$Fe(OH)_3$ composite NPs. PAA/CaP/$Fe(OH)_3$ composite NPs were collected by centrifugation at a speed of 8000 rpm for 5 min and washed two times with DI water. The PAA/CaP/$Fe(OH)_3$ composite NPs were calcined at 600 ˚C for 6 h under Ar atmosphere to gain carbon/CaP/Fe_3O_4 composite NPs.

Synthesis of 70 ± 20 nm, 140 ± 20 nm, and 200 ± 20 nm carbon/CaP/Fe_3O_4 composite NPs: PAA aqueous solution (50, 100, or 200 μL, 0.2 g/mL), $Ca(OH)_2$ (3, 6, or 12 mg) was added into DI water (10 mL) in a 50 mL flask, respectively. After stirring for 30 min, 20 mL of IPA was dropwise added into the mixture. After another 30 min, $FeCl_2$ solution (5, 10 or 20 mg of $FeCl_2$ in 0.2 mL DI water) was injected and stirring for 2 h. Then, $(NH_4)_2HPO_4$ (3.55, 7.1, or 14.2 mg in 0.2 mL DI water) was added four times for every 30 min intervals. The reaction was carried out overnight with continuous stirred at room temperature to obtain PAA/CaP/$Fe(OH)_3$ composite NPs. After centrifugation at a speed of 8000 rpm for 5 min and washed two times with DI water. The PAA/CaP/$Fe(OH)_3$ composite NPs was calcined at 600 ˚C for 6 h under a

high-purity argon atmosphere to gain carbon/CaP/Fe_3O_4 composite NPs with different diameters (70 ± 20, 140 ± 20, and 200 ± 20 nm).

DOX loading into carbon/CaP/Fe_3O_4 composite NPs and pH/NIR dual-responsive controlled release: UV-vis spectroscopy was used to determine the amount of loaded DOX in the carbon/CaP/Fe_3O_4 composite NPs. 1 mg of carbon/CaP/Fe_3O_4 composite NPs and 100 μL of DOX solution (10 mg mL^{-1}) was mixed in 2 mL of DI water and stirred for 48 h at room temperature. DOX loaded carbon/CaP/Fe_3O_4 composite NPs was obtained by centrifugation and washed once with DI water to get rid of the DOX adsorbed on the surface. The quantity of original DOX and the supernatant were detected by the UV-vis spectrophotometer at 490 nm. The DOX loading efficiency (LE) can be calculated by using equation 1:

$$LE\ (\%) = [m_{(original\ DOX)} - m_{(DOX\ in\ supernatant)}] / m_{(original\ DOX)} \quad (1)$$

Drug release from DOX loaded carbon/CaP/Fe_3O_4 composite NPs: To assess the in vitro DOX release from DOX-loaded carbon/CaP/Fe_3O_4 composite NPs, 3 mL of DOX loaded composite NPs was equally divided into three centrifuge tubes and centrifuged. Two of them were dispersed into 1 mL of phosphate buffered saline (PBS, pH = 5.3 and 7.4) and kept releasing in water bath at 37 ^oC without laser irradiation. To prove that the laser irradiation can promote the drug release, another (pH = 5.3) was exposed to the 808 nm NIR light at a power density of 2 W cm^{-2} at

selected time intervals. At desired time intervals, the supernatant was taken by centrifuging and the amount of DOX was measured by UV-vis spectrometer at 490 nm.

The photothermal effect of carbon/CaP/Fe_3O_4 composite NPs: First, the photothermal effect of carbon/CaP/Fe_3O_4 composite NPs was measured in aqueous solution. Briefly, carbon/CaP/Fe_3O_4 composite NPs (1 mL) with various concentrations were exposed to the 808 nm NIR laser (power density 2 W cm^{-2}) for 5 min. The temperature was recorded every 30 s. DI water was also irradiated with the NIR laser light as comparison. Subsequently, the photothermal effect in the cell level was analyzed by using calcein AM staining method. Calcein AM can only penetrate in live cells and emit green fluorescence. The cells were seeded in a 24-well plate (2.5×10^4 cells per well) for 24 h. Then, the cells were divided into four groups: group 1 with PBS only; group 2 incubated with carbon/CaP/Fe_3O_4 composite NPs (25 μg mL^{-1}); group 3 incubated with NIR; group 4 incubated with both composite NPs and NIR laser. The NIR laser used in this experiment was 2 W cm^{-2} and the irradiation time was 5 min. After all the treatment, the cells were finally stained with calcein AM.

Cell cytotoxicity in vitro: The cytotoxicity of empty carbon/CaP/Fe_3O_4 composite NPs, free DOX and DOX loaded carbon/CaP/Fe_3O_4 composite NPs were evaluated by standard 3-(4,5-

dimethylthialzol-2-yl)-2,5-diphe-nyltetrazo-lium bromide (MTT) assays. Hela cells were seeded in a 96-well plate at a density of 2.5×10^4 per well and incubated in the atmosphere of 95% air and 5% CO_2 at 37 ^oC with 10% fetal bovine serum for 24 h. Then various concentrations of empty carbon/CaP/Fe_3O_4 composite NPs, free DOX and DOX loaded carbon/CaP/Fe_3O_4 composite NPs were added, respectively. In another plate, the suspensions of carbon/CaP/Fe_3O_4 composite NPs with different concentrations were added into Hela cells with the 808 nm NIR laser irradiation for 5 min. One row of a 96-well plate was added culture medium only to be a blank control. Incubated for 24 h, 20 μL of 5 mg mL^{-1} MTT solution was added to each well for another 4 h incubation. Then, the medium was replaced with DMSO (150 μL) to dissolve the MTT formazan crystals. The cell viability can be calculated by using equation 2:

$$\text{Cell viability (\%)} = \text{Abs}_{(\text{test cells})}/\text{Abs}_{(\text{reference cells})} \times 100\% \quad (2)$$

MRI of carbon/CaP/Fe_3O_4 composite NPs: Firstly, different concentrations (0, 0.09, 0.19, 0.37, 0.75, 1.5 mg mL^{-1}) of carbon/CaP/Fe_3O_4 composite NPs suspending in water were prepared and the corresponding values of transverse relaxation time T_2 were recorded. The T_2 relaxivity (r_2) was determined by a linear fitting of the inverse relaxation times as a functional carbon/CaP/Fe_3O_4 multicomposite NPs

concentration. Then, Hela cells were seeded with various concentrations of carbon/CaP/Fe_3O_4 composite NPs in a 24-well plate at a density of 2.5 × 10^4 per well in the cell culture at 37 ^oC for 24 h. Washed with PBS for three times, the cells were suspended in 1 mL PBS for MRI. T_2-weighted MRI of the cell solutions were carried out by a 1.2 T MRI instrument (Shanghai Huantong Corporation HT-MRSI50-50KY).

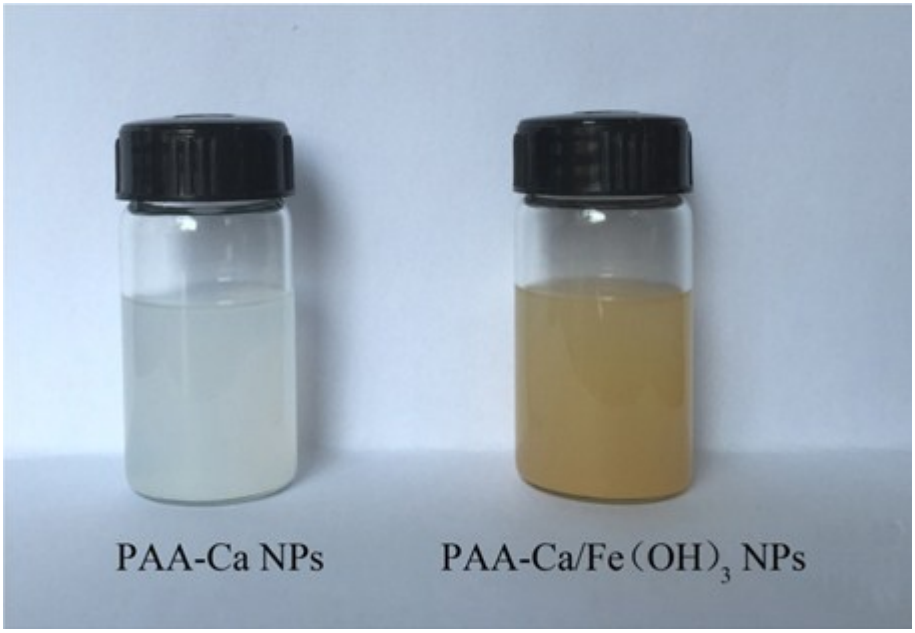

Fig. S1 Photographs of PAA-Ca NPs and PAA-Ca/$Fe(OH)_3$ NPs.

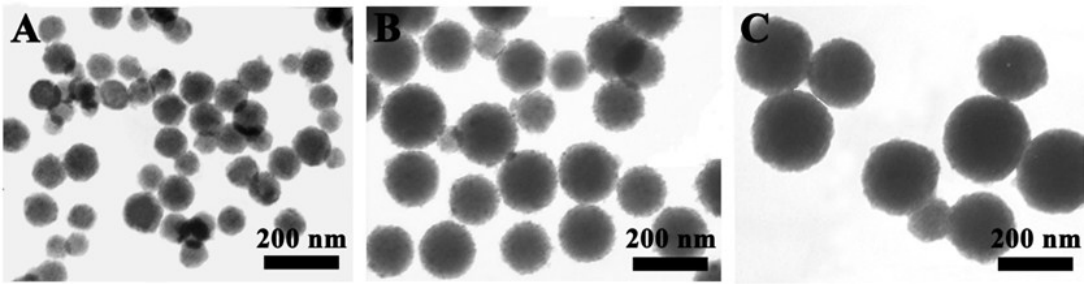

Fig. S2 TEM images of carbon/CaP/Fe_3O_4 composite NPs with different diameters (A-C) 70 ± 20 nm, 140 ± 20 nm, and 200 ± 20 nm.

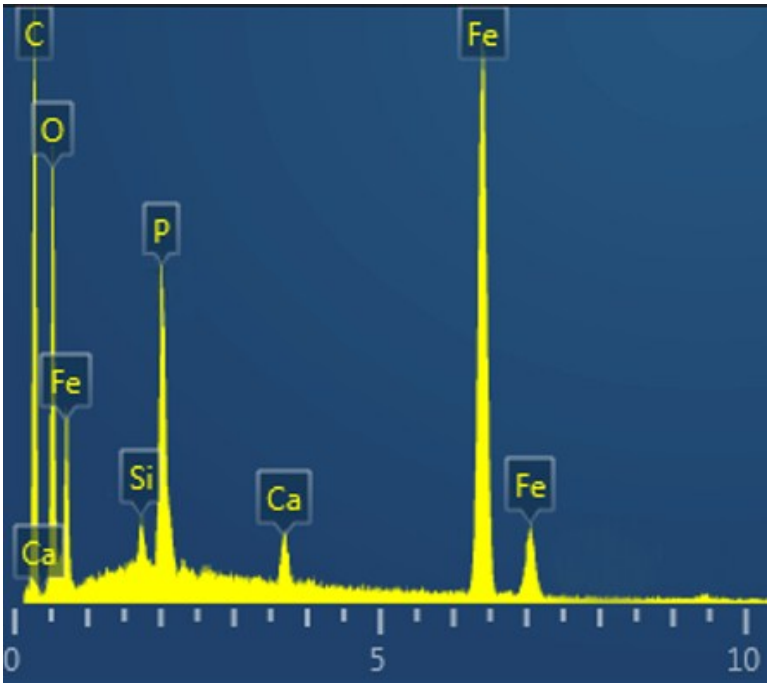

Fig. S3 Energy dispersive X-ray spectrum of carbon/CaP/Fe_3O_4 composite NPs

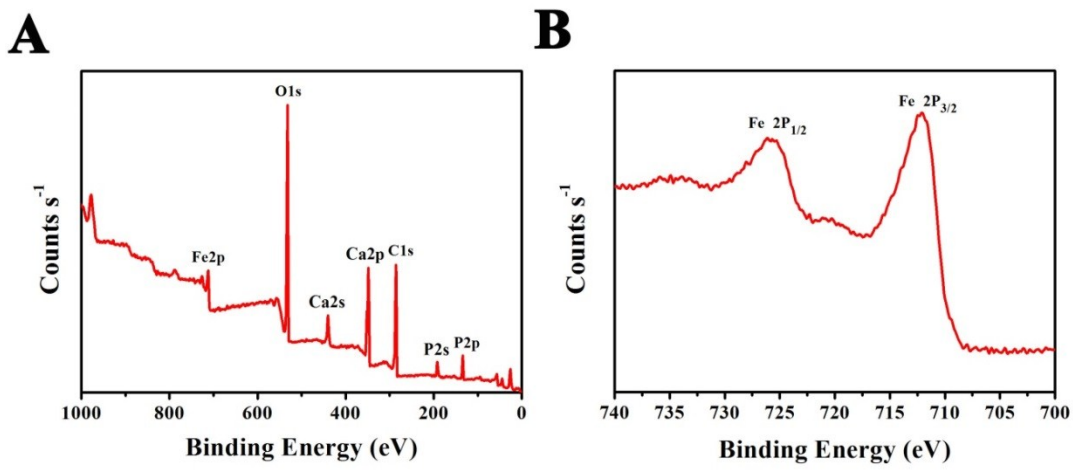

Fig. S4 X-ray photoelectron spectroscopy spectrum of the carbon/CaP/Fe_3O_4 composite NPs: (A) wide scan spectrum and (B) Fe 2p.

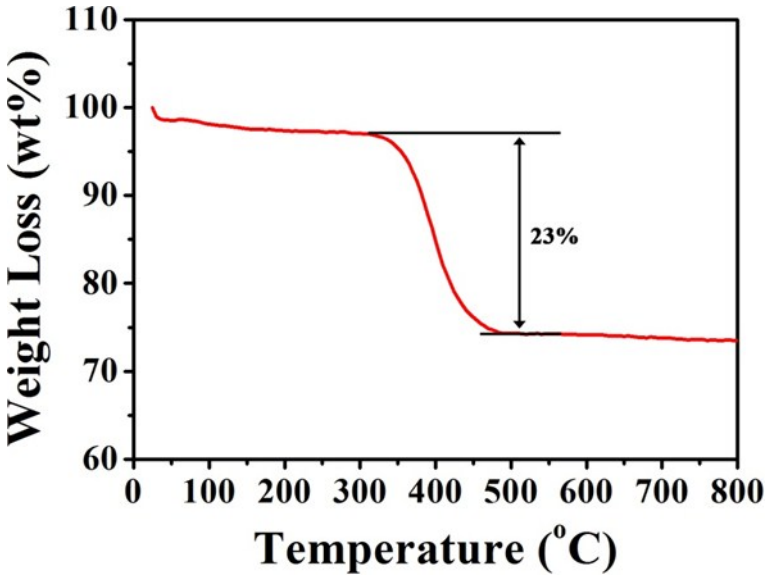

Fig. S5 TG curve of carbon/CaP/Fe_3O_4 composite NPs.

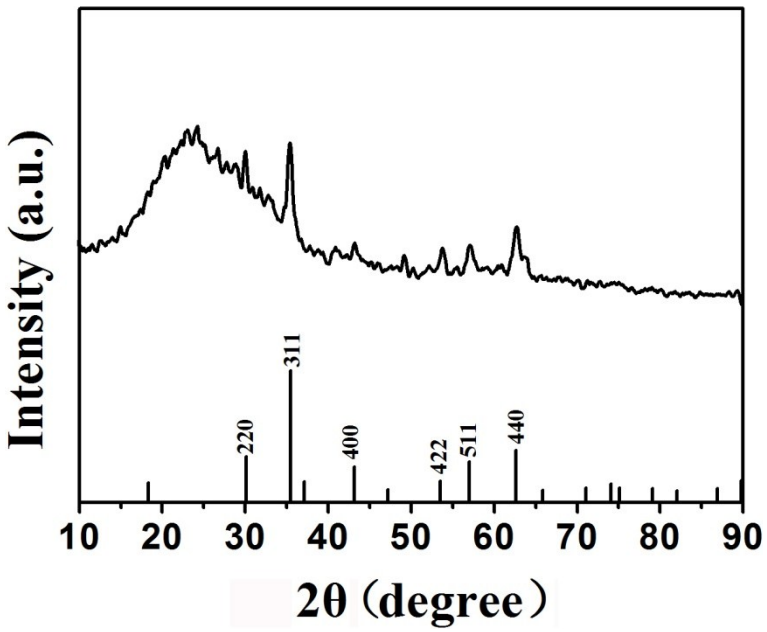

Fig. S6 XRD pattern of carbon/CaP/Fe_3O_4 composite NPs.

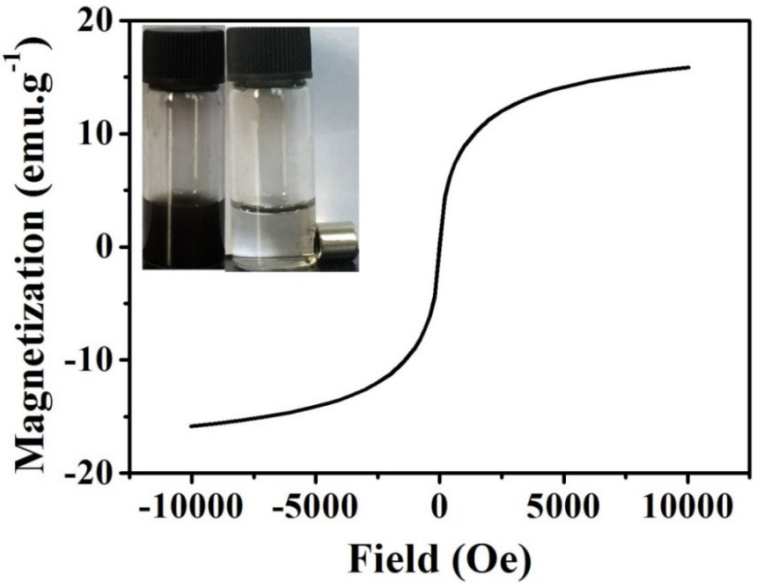

Fig. S7 Hysteresis loop measurement of carbon/CaP/Fe_3O_4 composite NPs at 300 K, inset: photographs of carbon/CaP/Fe_3O_4 composite NPs solutions without (left) and with (right) a magnet.

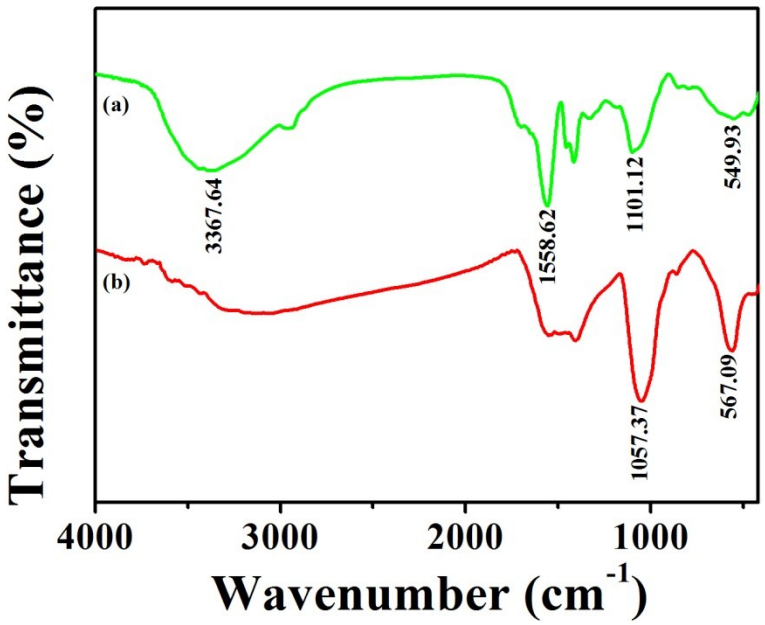

Fig. S8 FTIR spectra of (a) PAA/CaP/$Fe(OH)_3$ composite NPs and (b) carbon/CaP/Fe_3O_4 composite NPs.

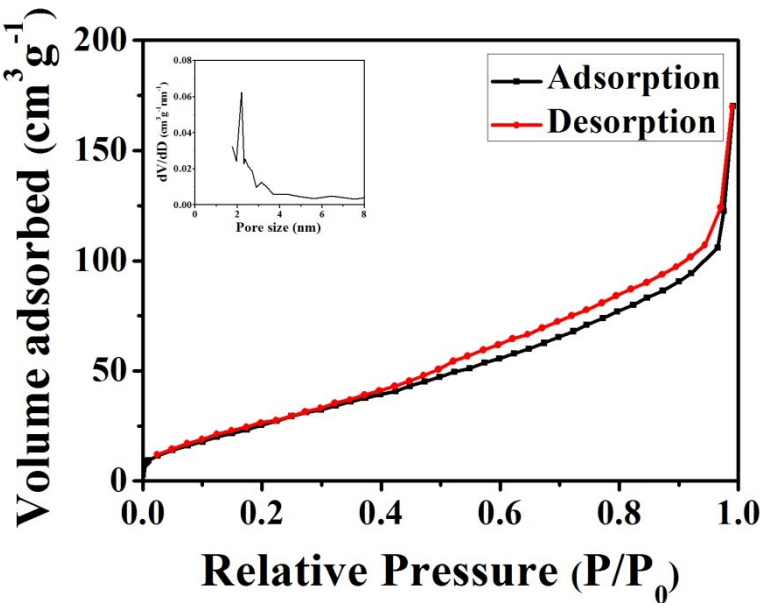

Fig. S9 The N_2 adsorption/desorption isotherm and the pore size distribution curve (inset) of carbon/CaP/Fe_3O_4 composite NPs.

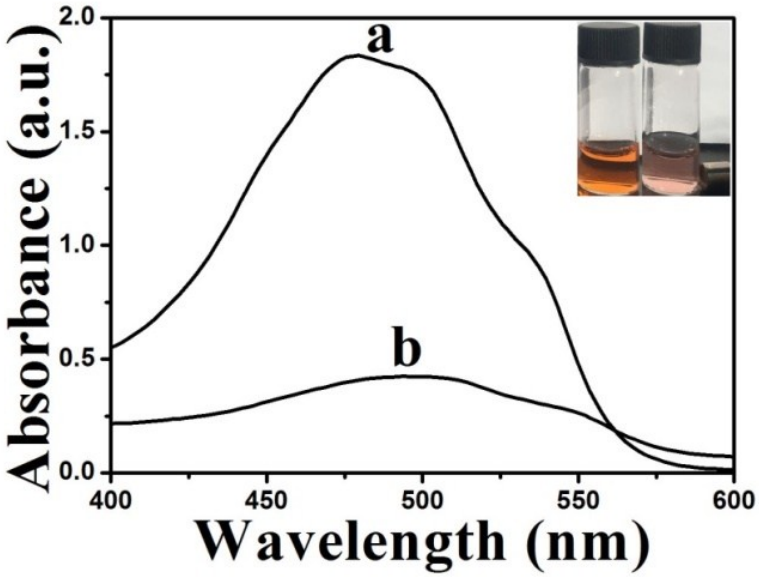

Fig. S10 UV-vis absorption spectra of DOX solutions (a) before and (b) after interacting with carbon/CaP/Fe_3O_4 composite NPs; the inset is the photograph of DOX loaded NPs without (left) and with (right) an external magnet for magnetic separation.

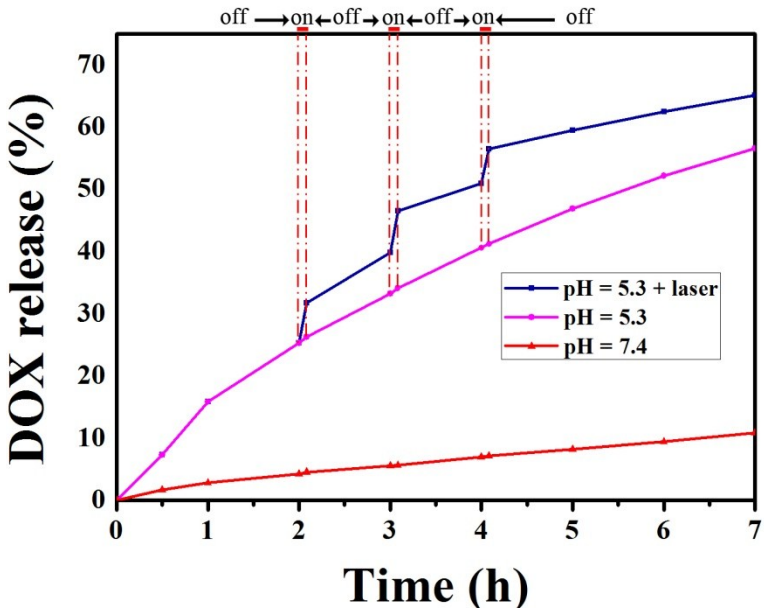

Fig. S11 DOX release profiles of DOX loaded carbon/CaP/Fe_3O_4 composite NPs in PBS at pH values of 7.4, 5.3 and 5.3 with periodic laser on/off irradiation (808 nm, 2 W cm^{-2}) at 37 ºC.

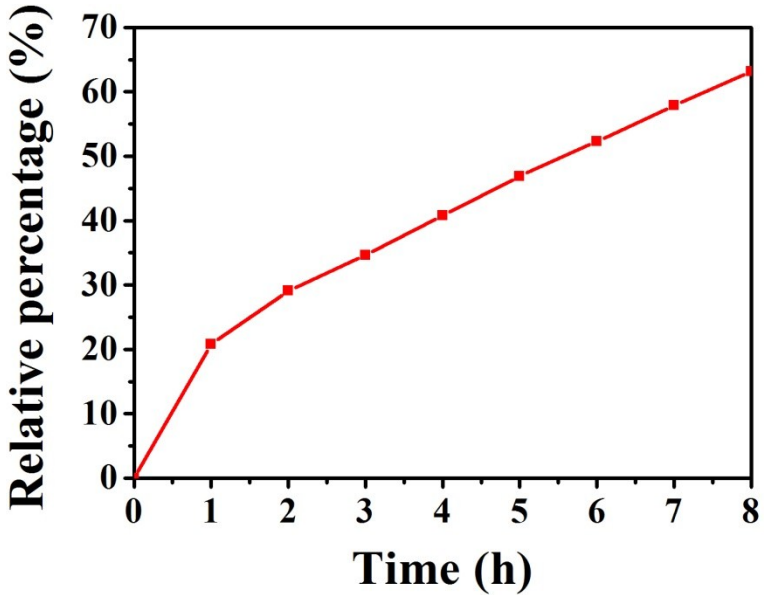

Fig. S12 The Ca^{2+} content profile for the carbon/CaP/Fe_3O_4 composite NPs in the PBS (pH 5.3) at different time points at 37 ºC.

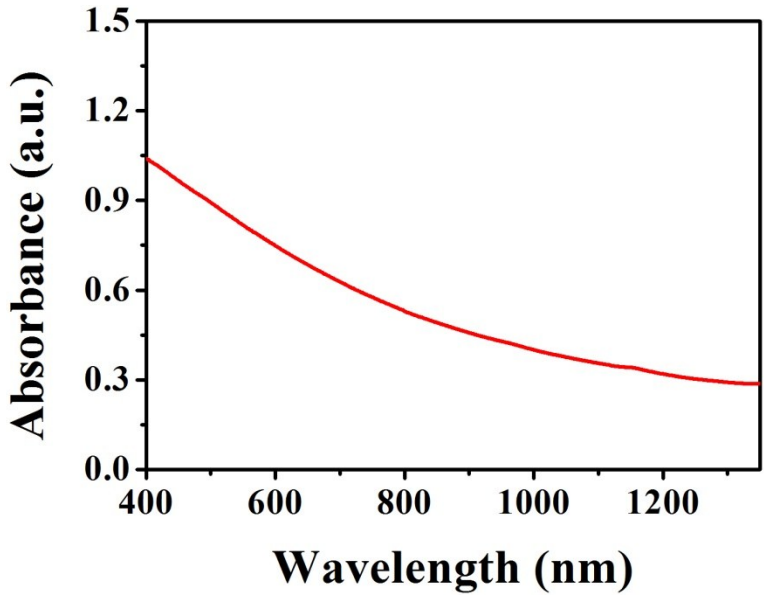

Fig. S13 UV-Vis-NIR absorption spectrum of carbon/CaP/Fe_3O_4 composite NPs.

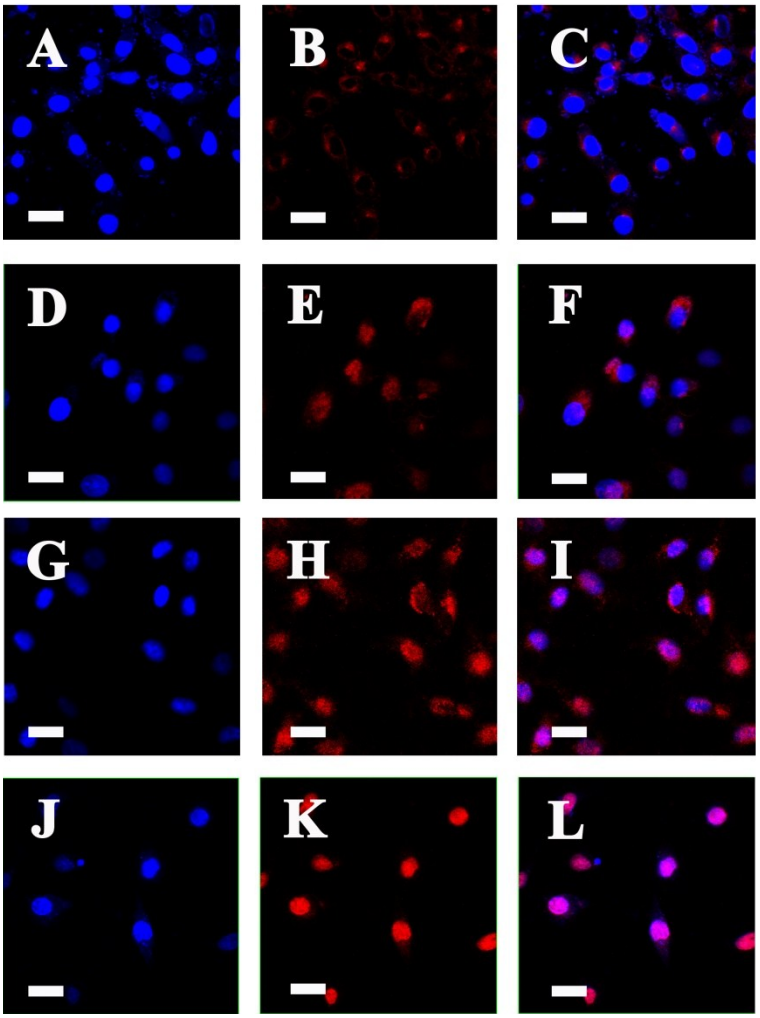

Fig. S14 CLSM images of HepG-2 cells incubated with DOX-loaded carbon/CaP/Fe_3O_4 composite NPs for 1 h (A-C), 3 h (D-F), 15 h (G-I) and 24 h (J-L) at 37 ^oC, respectively. Each series can be classified to cell nucleus (being dyed in blue by Hoechst 33342), DOX-loaded carbon/CaP/Fe_3O_4 composite NPs and the merged images of both above, respectively. All scale bars are 20 μm.