Supporting Information

Experimental section

Materials and Instrumentation: Anhydrous iron (II) chloride, anhydrous iron(III) chloride, calcium nitrate, monopotassium phosphate, ethylene glycol, acetone, ethanol and citric acid were all purchased from Beijing Chemicals (Beijing, China). $AgNO_3$ (more than 99%), PVP (powder, average Mr E 29,000 or 55,000), Na_2S, alendronate sodium trihydrate (97%) and $HAuCl_4$-$3H_2O$, 99.9+% were all purchased from Sigma Aldrich (Boston, MA, USA). 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), thioctic acid were obtained from Alfa Aesar. N-hydroxysulfosucnimide sodium salt (sulfo-NHS) was purchased from Pierce Biotechnology. Nanopure water (18.2MΩ/cm, Millpore Co., USA) was used in all experiments and to prepare all buffers.

Fourier transform infrared spectrophotometer (FTIR) analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. Scanning electron microscopy (SEM) images were obtained with a Hitachi S-4800 FE-SEM. Transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. UV-vis spectroscopy was carried out with a JASCO V-550 UV/vis spectrometer. X-ray measurements were performed on a Bruker D8 FOCUS Powder X-ray Diffractometer (XRD) using Cu Ka radiation. Fluorescence measurements were carried out on a JASCO FP-6500 spectrofluorometer. A CWdiode laser (LSR808NL-2000) with wavelength of 808 nm was used for the laser irradiation experi-ment.

Synthesis of Gold Nanocages (AuNCs)**:** AuNCs were synthesized by means of the galvanic replacement reaction between Ag nanocubes (Fig. S1a, ESI†) and chloroauric acid ($HAuCl_4$) according to a previously reported method.[1] For all of the experiments, we used AuNCs of 50±10 nm

in edge length together with a pore size of 5-10 nm. As shown in Fig. S1b, the maximum absorption of the prepared AuNCs was at 795 nm. Briefly, 500 μL of the Ag nanocubes (3 nM) was added to 5 mL of deionized water containing poly (vinyl pyrrolidone) (PVP, 1 mg/mL) hosted in a 50 mL flask under magnetic stirring and then heated to boil for 10 min. In the meantime, an aqueous solution of $HAuCl_4$ (0.5 mM) was prepared. The $HAuCl_4$ was added to the flask at a rate of 45 mL/h until the solution had an optical extinction peak at 795 nm as confirmed by UV spectroscopy. The solution was refluxed for another 30 min until the color of the reaction was stable. Once cooled to room temperature, the sample was centrifuged and washed with saturated NaCl solution to remove AgCl and with water several times to remove PVP and NaCl.

Synthesis of Bisphosphonate Functionalized Gold Nanocages (AuNCs-BP)**:** AuNCs-BP were prepared by a two-step method. We firstly replace the PVP on nanocages with the thioctic acid to obtain AuNCs-COOH. The resultant carboxyl unit on the surface was activated by EDC and sulfo-NHS and subsequently treated with alendronate to obtain AuNCs-BP. The AuNCs-BP were purified for further uses through several cycles of centrifugation, decantation and redispersion. The covalently grafted bisphosphonate group onto AuNCs was evidenced by FTIR spectroscopy (Fig. S1c, ESI†). The strong –CO–NH– (1640 cm^{-1}, 1550 cm^{-1}) vibrations and the characteristic P=O stretch mode (1226 cm^{-1}) in the FTIR spectrum indicated that bisphosphonate group was covalently grafted to the AuNCs surface successfully.[2] The energy-dispersive spectroscopy (EDS) analysis also supported the presence of S and P in the as-prepared nanoparticles (Fig. S1d, ESI†).

Synthesis of Calcium Phosphate Coated Magnetic Nanoparticles (Fe_3O_4@CaP): The successful preparation of nontoxic Fe_3O_4@CaP was achieved according to previously reported procedure with a minor modification and was characterized by TEM, XRD analyses, and EDS analyses.[3] Here, iron (II)

chloride and iron (III) chloride were dissolved in 25 ml of deoxygenated water in the presence of 0.4 M hydrochloric acid. This solution was added dropwise at 0.5 ml/min to a 250 ml solution of 1.5 M sodium hydroxide under nitrogen gas flow. This solution was centrifuged at 5000 rpm and the supernatant decanted. The particles were dispersed in double deionized water while vortexing followed by sonication. This was repeated three times whereby a black solution of magnetite was obtained. The particles were sterilized through dispersion in ethanol once, centrifuged at 5000 rpm, and decanted before suspension in double deionized water. Calcium phosphate (CaP) coatings were produced on magnetic nanoparticles in the presence of citric acid capping agents according to a previous report. Surfactants have been shown to have a stabilizing effect on magnetic nanoparticles. Citric acid were used with the motive to disperse the magnetic nanoparticles so that an effective CaP coating could be created. Into a 20 ml solution of double deionized water, 2.67 mmol of calcium nitrate was added. This solution was dissolved while magnetically stirring and the pH was adjusted to 10 by the addition of ammonium hydroxide.1.147 mmol of iron oxide nanoparticles and 0.18 mmol of critic acid were then added to the calcium solution. Separately, a 20 ml solution was prepared using 1.6 mmol of monopotassium phosphate whereby the pH was adjusted to 10 using ammonium hydroxide. This solution was added dropwise to the calcium solution. Then, the mixture was moved to a PTFE hydrothermal unit and heated for 9 h at 70 ◦C. After cooling, particles were centrifuged (5000 rpm) and the aqueous component was decanted for dispersion in ethanol for sterilization purposes as described above. After subsequent sonication, the coated magnetic nanoparticles were again centrifuged and the solvent decanted. This was repeated for a total of three times before their dispersion in double deionized water.

The TEM image revealed the diameter of the Fe_3O_4@CaP nanoparticle was 8-15 nm. The iron

cores were seen as dark spheres, surrounded by CaP coating (Fig. S2a, ESI†). Wide-angle XRD patterns showed that Fe_3O_4@CaP exhibited the peaks of premature amorphous CaP (Fig. S2b, ESI†).[3] EDS analyses showed that the surfaces of Fe_3O_4@CaP predominantly contained Ca and P which belonged to CaP minerals (Fig. S2c, ESI†).

Doxorubicin Loading and Fe_3O_4@CaP Capping: The purified linker-AuNCs was stirred vigorously (vortex stirrer) in a solution of doxorubicin (DOX) (0.2mM) in PBS solution (10 mM, pH 7.4) for 24 h, followed by centrifuging and washing with PBS solution (10 mM, pH 7.4) to remove molecules from the exterior surface of the material. Then, Fe_3O_4@CaP were added to the suspension, and the mixture was stirred for another 12 h, followed by centrifuging (7000rpm) and repeated washing with PBS solution (10 mM, pH 7.4). All the washing solutions were collected, and the loading of DOX was calculated from the difference in the concentration of the initial and left DOX.

Controlled Release Experiments: In vitro release profiles of DOX from Fe_3O_4@CaP capped AuNCs were examined in the aqueous buffer solutions (pH 7.4 PBS buffer, pH 4.5 PBS buffer and pH 4.5 PBS buffer with 1W 808nm NIR irradiation). Fe_3O_4@CaP capped DOX loaded AuNCs (AuNCs-DOX) was dispersed in each release medium. The release medium was shaken at a speed of 150 rpm. At predetermined time intervals, the release samples was centrifuged, the supernatants were withdrawn and replaced with an equal volume of the fresh medium. The concentration of released DOX was determined by measurement of UV-visible spectra at 480 nm based on the standard curve.

Cell Culture: Human breast cancer MCF-7 cells (Human lung adenocarcinoma A549 cells) were cultured in 25 cm^2 flasks in Dulbecco's Modified Eagle's Medium DMEM (Gibco) containing 10% (v/v) fetal bovine serum (Gibco) at 37°C in an atmosphere of 5% (v/v) CO_2 in air. The media were changed every three days, and the cells were passaged by trypsinization before confluence.

Fluorescence Imaging: MCF-7 cells were seeded in a 24-well plate and cultured for 24 h. The cell medium was removed, and then cells were incubated with 0.5 mL of fresh cell medium containing 30 μg of AuNCs-DOX for 1.5 h and 4 h. After that, the medium was replaced by cell medium with Hoechst .Cell imaging was then carried out after washing cells with PBS.

In Vitro Cytotoxicity Assay: For studying the cytotoxicity, MCF-7 cells (A549 cells) were seeded in a 96-well plate at a density 10^4 cells/well for 24 h at 37°C in 5% CO_2. Then, the cells were treated with free DOX, Fe_3O_4@CaP capped AuNCs and AuNCs-DOX at desired concentration. After incubation for 24 h, cell viabilities were tested by standard MTT (3-(4, 5)-dimethylthiahiazo-2-yl)-2, 5-diphenyltetrazolium bromide) assay. To confirm that the nanoparticles could efficiently kill cancer cell under laser irradiation, MCF-7 cells (A549 cells) were cultured in 96-well plate for 24 h. After that, the medium was replaced by cell medium with AuNCs-DOX or free DOX at proper concentration. After maintained for 6 h, the plates were photo-irradiated by a laser lamp (λ = 808 nm, 1 W) for 5 min. Cell viabilities were also tested by standard MTT assay.

References

1. S. E. Skrabalak, L. Au, X. Li, Y. Xia, *Nat. Protoc.*, **2007**, *2*, 2182-2190.
2. R. D. Ross, R. K. Roeder, *J. Biomed. Mater. Res.*, **2011**, *99A*, 58-66.
3. R. A. Pareta, E. Taylor, T. J. Webster, *Nanotechnology*, **2008**, *19*, 265101.

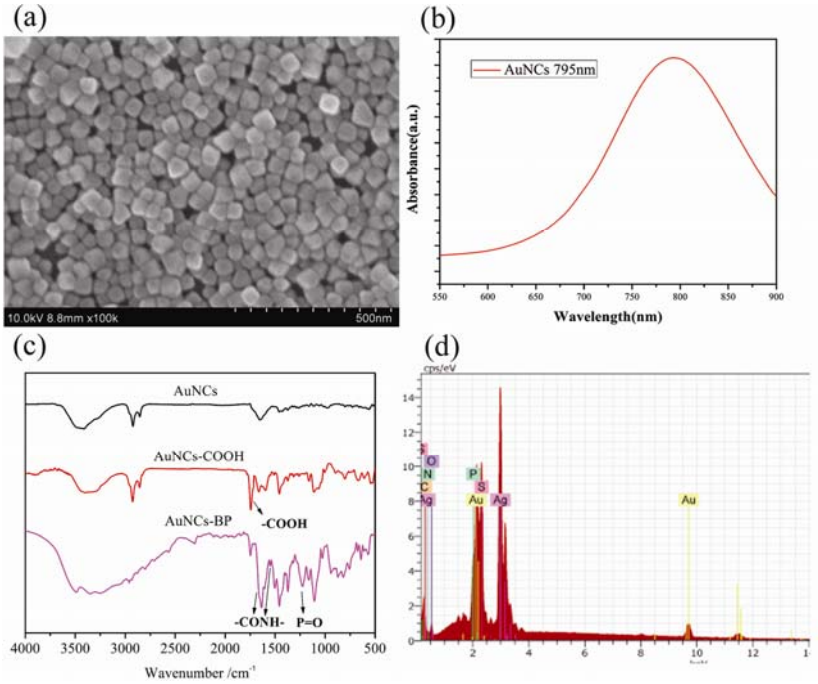

Fig. S1 (a) SEM image of Ag nanocubes. (b) UV-vis absorption spectra of the Au nanocages. (c) FTIR spectra of AuNCs, AuNCs-COOH and AuNCs-BP. (d) SEM-associated EDS spectra of the bisphosphonate functionalized AuNCs.

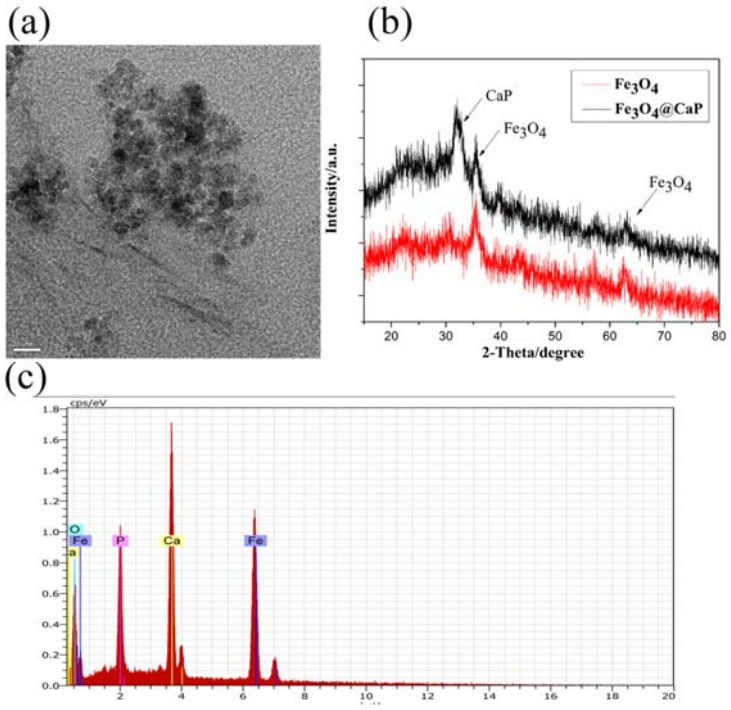

Fig S2 (a) TEM image of Fe_3O_4@CaP. (b) Wide-angle XRD patterns of magnetic nanoparticles before and after calcium phosphate coating. (c) SEM-associated EDS spectra of Fe_3O_4@CaP.

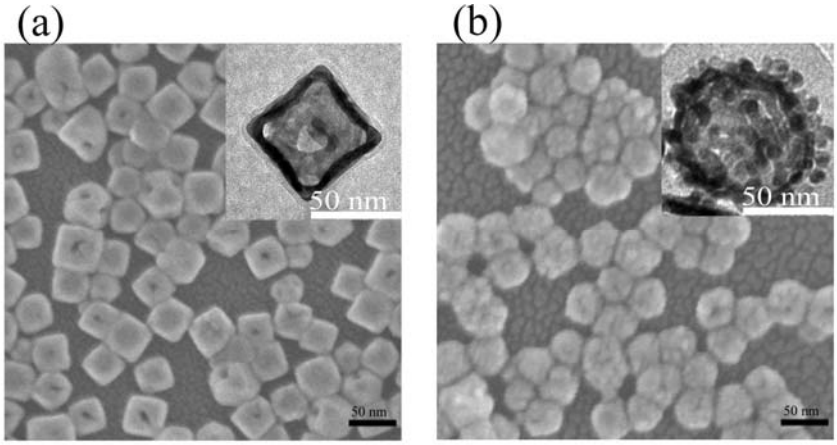

Fig. S3 (a) SEM image of AuNCs. Inset: TEM image of AuNCs. (b) SEM image of Fe_3O_4@CaP capped AuNCs. Inset: TEM image of Fe_3O_4@CaP capped AuNCs.

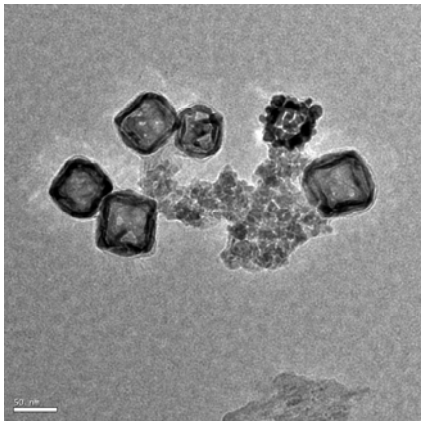

Fig. S4. TEM image of Fe_3O_4@CaP capped AuNCs incubated in PBS buffer (pH 4.5) for a few hours.

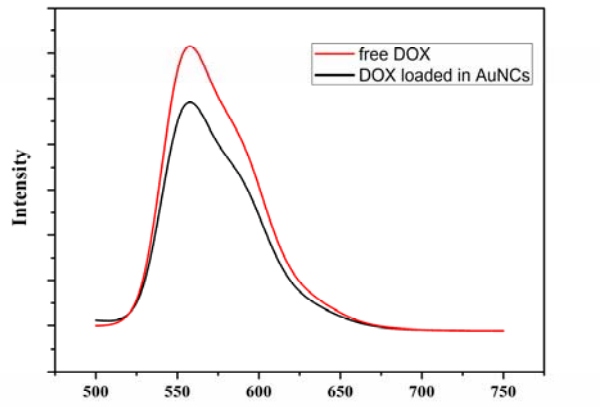

Fig. S5 Fluorescence spectra of free DOX (2.75μg/ml) and AuNCs-DOX 50μg/ml (DOX=2.75μg/ml).

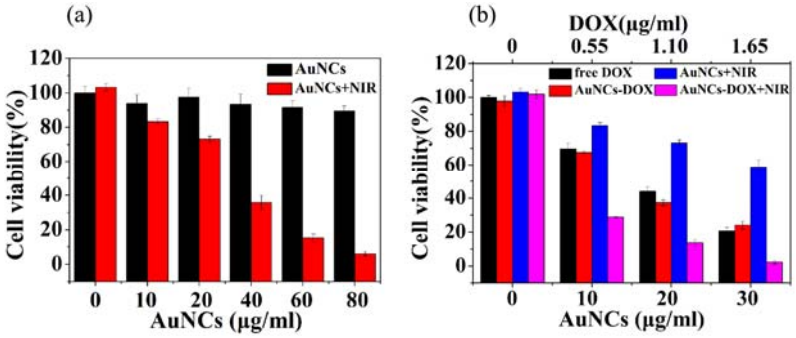

Fig. S6 (a) Viability of A549 cell incubated for 24 h with different concentrations of Fe_3O_4@CaP capped AuNCs with or without 5-min NIR irradiation (1 W, 808 nm). (b) A549 cell viabilities after a 24 h incubation with different concentrations of free DOX, Fe_3O_4@CaP capped AuNCs with 5-min NIR irradiation (1 W, 808 nm) and AuNCs-DOX with or without 5-min NIR irradiation (1 W, 808 nm).