Electronic Supplementary Information (ESI)

Near-infrared light and pH-responsive Au@carbon/calcium phosphate nanoparticles for imaging and chemo-photothermal cancer therapy of cancer cells

Huiyuan Wang,[a] Manjie Zhang,[a] Lingyu Zhang,[a] Shengnan Li,[a] Lu Li,[a] Xiliang Li,[a] Min Yu,[a] Zhongcheng Mou,[a] Tingting Wang,*[b] Chungang Wang*[a] and Zhongmin Su[a]

a Department of Chemistry, Northeast Normal University
Changchun, 130024 (P.R. China)

b School of Chemistry & Environmental Engineering
Changchun University of Science and Technology
Changchun, 130022 (P.R. China)
E-mail: wangcg925@nenu.edu.cn, wangtt@cust.edu.cn

Experimental Section

Chemicals and Reagents: Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$), polyacrylic acid (PAA, $M_W \approx 1800$), trisodium citrate, doxorubicin and hydrochloride (DOX) were purchased from Sigma (USA). Isopropyl alcohol (IPA), calcium hydroxide ($Ca(OH)_2$), sodium hydrogen phosphate (Na_2HPO_4) were purchased from Sinopharm Chemical Reagent Beijing Co, Ltd. Deionized water (DI water) was used in all experiments.

Characterization: Transmission electron micrographs (TEM) were taken by JEOL-2100F transmission electron microscope under a 200 kV accelerating voltage. Scanning electron microscopy (SEM) images and the energy dispersive X-ray (EDX) spectrum were carried out with a JEOL JSM-7610F scanning electron microscope. Fourier transform infrared (FTIR) spectra were obtained on a Magna 560 FTIR spectrometer (Nicolet, USA). Fluorescence microscopy was operated on Olympus DP73. The UV-Visible-NIR spectra were recorded at room temperature on a Japan JASCO V-570 spectrometer fluorescence spectrophotometer. X-Ray photoelectron spectra (XPS) were measured by an ECSALAB 250 using non-mono-chromatized Al-Kα radiation. A CW diode laser (LSR808H) with wavelength of 808 nm was used for the laser irradiation experiment. N_2 sorption analysis was performed by an intelligent gravimetric analyzer Autosorb-iQ (Quantachrome). The size and zeta potential of the nanoparticles (at a concentration of 0.05 mg mL^{-1}) were measured by dynamic light scattering (DLS) using a Malvern Zeta Sizer (Nano-ZS). Confocal laser scanning microscopy (CLSM) was observed by means of an Olympus Fluoview FV1000. Inductively coupled plasma atomicemission spectroscopy (ICP-AES) was measured with Leeman ICP-AES Prodigy. The temperature change of the solution was recorded by a T460SC, infrared camera (FLIR, Sweden). X-ray computed tomography (CT) images were obtained by using a SIEMENS SOMATOM Sensation 64 and the Hounseld Unit (HU) variations were determined by using a syngo CT 2009S instrument (Siemens, Berlin).

Synthesis of Au@C/CaP core-shell NPs

Monodispersed Au NPs about 50 nm were prepared by reduction of tetrachloroauric

acid by sodium citrate.[1] In a 500 mL of flask, PAA aqueous solution (300 μL, 0.2 g mL^{-1}) and 24 mg of $Ca(OH)_2$ were mixed in 30 mL of DI water with magnetic stirring for 20 min. Then, 25 mL of the as-prepared AuNPs were added into the solution to form a suspension. Afterwards, 60 mL of IPA was dripped into the suspension under magnetic stirring to form the Au@PAA-Ca core-shell NPs. With continuous stirring for 5 h, Na_2HPO_4 solution (76.8 mg of Na_2HPO_4 in 500 μL DI water) was added into the flask. The reaction mixture was reacted for 12 h with continuous stirring at room temperature to obtain the Au@PAA/CaP NPs. The Au@PAA/CaP NPs were calcined at 500 °C for 3 h under a high-purity argon atmosphere to gain the core-shell Au@C/CaP NPs.

Synthesis of core-shell Au@C/CaP NPs with 30, 70 and 100 nm C/CaP shell thicknesses

In a 500 mL of flask, PAA aqueous solution (100, 200, and 300 μL), $Ca(OH)_2$ (8.0, 16.0, and 24.0 mg) were mixed in 30 mL of DI water with magnetic stirring for 20 min, respectively. Then, 25 mL of the as-prepared AuNPs were added into the solution to form a suspension. After that, 60 mL of IPA was dripped into the suspension under magnetic stirring. After stirring for 20 min, Na_2HPO_4 solution (25.6, 51.2 and 76.8 mg of Na_2HPO_4 in 500 μL DI water) was added into the flask under magnetic stirring at room temperature for 12 h to obtain Au@PAA/CaP core-shell NPs. Finally, the Au@PAA/CaP core-shell NPs were calcined at 500 °C for 3 h under a high-purity argon atmosphere to gain the Au@C/CaP core-shell NPs with different shell thicknesses (30, 70, and 100 nm).

DOX loading and pH/NIR dual-responsive controlled release in vitro

1 mg of Au@C/CaP NPs and 100 μL of DOX solution (10 mg mL^{-1}) was mixed in 3 mL of DI water and stirred for 48 h at room temperature. DOX-loaded Au@C/CaP NPs was obtained by centrifugation and washed three times with DI water to get rid of the DOX adsorbed on the surface. To evaluate the DOX loading efficiency (LE), the amount of original DOX and all the supernatants were determined by measuring the absorbance at 480 nm using a UV-Vis spectrophotometer. The LE of DOX was calculated by Equation (1) :

$$LE\ (\%) = [m_{(total\ DOX)} - m_{(DOX\ in\ supernatant)}]/m_{(total\ DOX)} \times 100\% \quad (1)$$

To assess of the DOX release from DOX-loaded Au@C/CaP NPs in vitro, 3 mL of DOX-loaded NPs was equally divided into three centrifuge tubes and centrifuged. Then, 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 °C without laser irradiation. The other sample in PBS (pH 5.3) was exposed to the 808 nm NIR light (2 W cm^{-2}) at selected time intervals to prove that the laser irradiation can promote the drug release. The supernatant was taken by centrifuging and the residual DOX was measured by UV-Vis spectrometer at 480 nm. To further prove the dissolution of CaP. The Au@C/CaP NPs (1.0 mg) were placed into pH 5.3 and 7.4 PBS buffer (4.0 mL), respectively. The supernatants were gathered at selected time intervals by centrifugation and tested by ICP-AES to measure the content of Ca.

Measurement of photothermal performance of Au@C/CaP NPs

To measure the photothermal performance of the Au@C/CaP NPs, the samples with different concentrations (0, 0.1, 0.2, 0.4, and 0.8 mg mL^{-1}) were prepared and exposed to the 808 nm NIR laser (power density 2 W cm^{-2}), or at a fixed concentration of 0.7 mg mL^{-1} with irradiation at various laser intensities. The samples were cyclically irradiated for 5 min each time, and the temperature was recorded every 30 s. Subsequently, The live/dead double-staining method was used to evaluate chemophotothermal therapy in cell levels. Calcein acetoxymethyl ester (Calcein AM) stained the live cells with green emission whereas propidium iodide (PI) dye stained the dead cells with red emission. The HeLa cells were seeded in 96-well plates (2.5×10^4 cells per well) and incubated for 24 h. Then, the cells were divided into four groups: group 1 with PBS only; group 2 incubated with Au@C/CaP NPs (25 μg mL^{-1}); group 3 incubated with NIR; group 4 incubated with both Au@C/CaP NPs and NIR laser. The NIR laser used in this experiment was 2 W cm^{-2} and the irradiation time was 5 min.

Investigatation of photostability and heating reproducibility of Au@C/CaP NPs

The photostability of Au@C/CaP NPs was investigated by monitoring their absorbance changes upon continuous 808 nm laser irradiation at a power of 2 W cm^{-2}

for different time points. Heating/cooling curve of the Au@C/CaP NPs at a concentration of 0.4 mg mL^{-1} was completed over 4 cycles. Each cycle includes 5 min of laser irradiation to generate heat with a cooling time of 16 min before the start of the next cycle. Temperatures were recorded immediately after 5 min of laser irradiation and following the 16 min of cooling period.

Cell uptake and cellular uptake of the Au in the Au@C/CaP NPs

The HeLa cells were seeded in 24 well plates with a clean cover-slip per well and incubated with 20 ug mL^{-1} of DOX-loaded Au@C/CaP NPs for 1, 2, 4 and 6 h in the 5% CO_2 environment at 37 °C. After incubation, the cell supernatant was discarded and the cell monolayer was washed with PBS (pH 7.4). Subsequently, HeLa cells were stained with 4,6-diamino-2-phenylindole (DAPI). The cover-slips were mounted onto a glass microscope slide and the cells were observed using a CLSM. The cells were washed with PBS buffer three times, trypsinized and harvested by centrifugation. The digestion of the cells was performed in aqua regia and the amount of gold uptake in the cells was then quantified using ICP-AES.

Cell cytotoxicity in vitro

The cell viability of empty Au@C/CaP NPs, free DOX, DOX-loaded Au@C/CaP NPs, Au@C/CaP NPs with NIR laser and DOX-loaded Au@C/CaP NPs with NIR laser was evaluated by Celltiter-Blue cell viability assay. HeLa cells were seeded into 96-well plates at a density of 2.5×10^4 cells per well, and incubated in DMEM containing fetal bovine serum (FBS), 100 units per mL of penicillin and 100 µg ml^{-1} of streptomycin at 37 °C for 24 h. Then, the medium was replaced by serum-free DMEM containing different concentrations of DOX-loaded Au@C/CaP NPs. After 24 h, the medium was removed, 0.1 mL fresh serum-free DMEM containing 10 µL of Celltiter-Blue reagent was added to each well and the plates were incubated for another 4 h at 37 °C. Finally, the fluorescence signal was measured by microplate reader (λ_{ex} = 560 nm, λ_{em} = 590 nm).The cell viability was calculated based on the equation (2):

$$\text{Cell viability (\%)} = [Abs_{(\text{test cells})} / Abs_{(\text{reference cells})}] \times 100\% \quad (2)$$

CT imaging of Au@C/CaP NPs in vitro

The CT images of Au@C/CaP NPs samples in PBS with various Au concentrations (0, 0.19, 1.16, 3.25, 5.23 and 8.27 mg mL^{-1}) were obtained by using a SIEMENS SOMATOM Sensation 64 with a tube voltage of 120 kV, an electrical current of 280 mA, and a slice thickness of 1.0 mm. Phantom images were treated by using a standard image viewer application to measure the mean HU variation of the acquired image depending on the Au concentration.

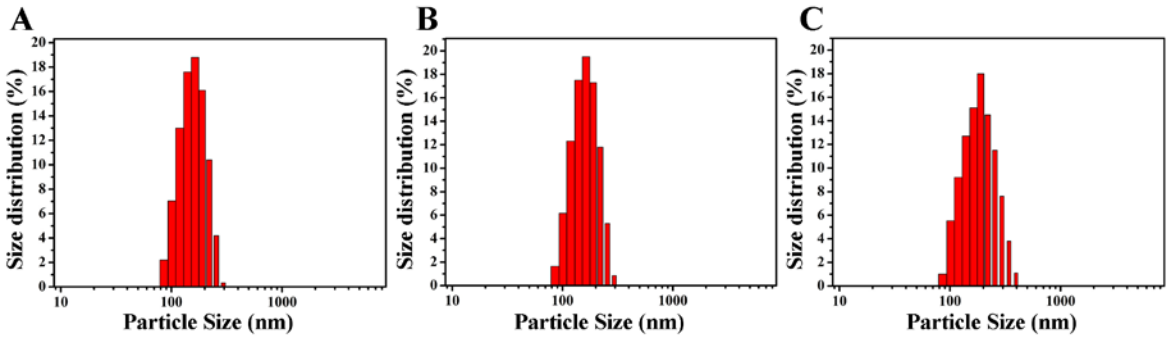

Figure S1. Size distributions of the Au@C/CaP NPs dispersed in water (A), PBS (pH = 7.4) (B) and culture medium with serum (C).

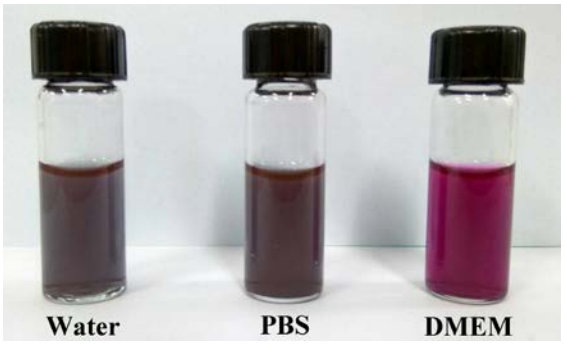

Figure S2. Photographs of the Au@C/CaP NPs in water, PBS buffer and culture medium with serum and they were stored for 12 h, respectively.

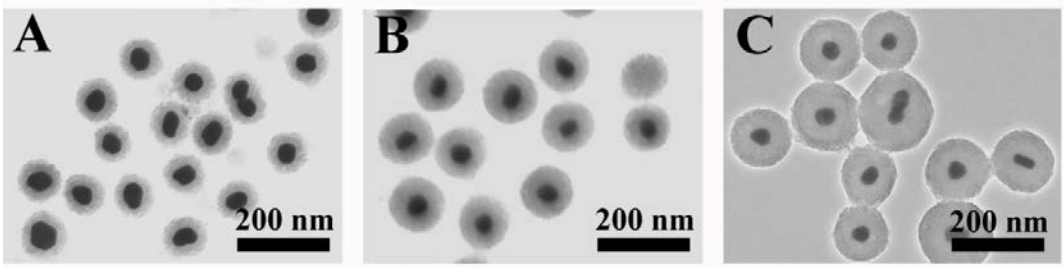

Figure S3. TEM images of Au@C/CaP NPs with different shell thicknesses (A-C) 30, 70, and 100 nm C/CaP, respectively.

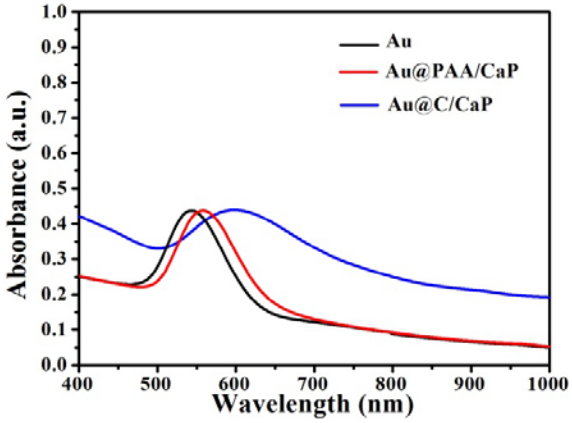

Figure S4. Vis-NIR absorption spectra of the Au (black), Au@PAA/CaP (red) and Au@C/CaP (blue).

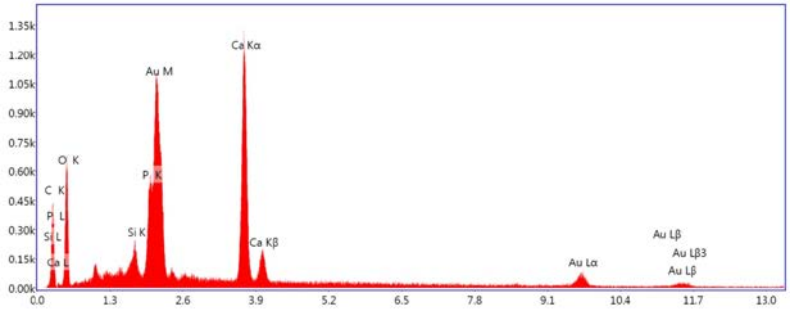

Figure S5. Energy dispersive X-ray spectrum of Au@C/CaP NPs.

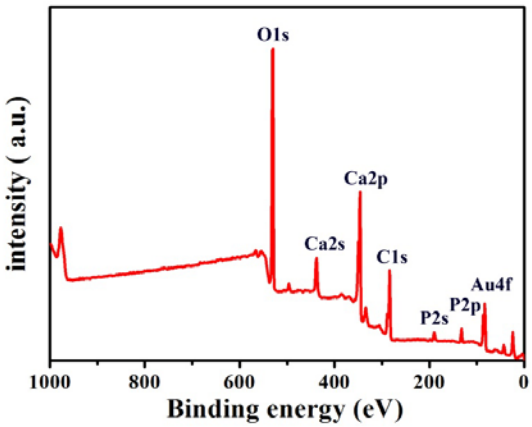

Figure S6. XPS of Au@C/CaP NPs.

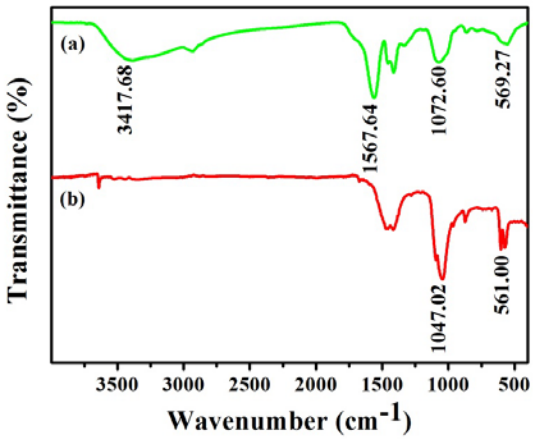

Figure S7. FTIR spectra of (a) Au@PAA/CaP NPs and (b) Au@C/CaP NPs.

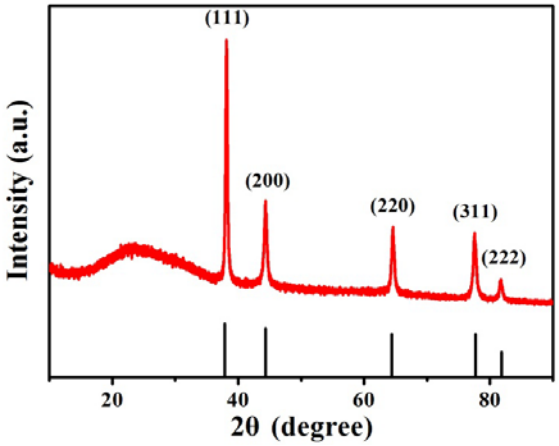

Figure S8. XRD pattern of Au@C/CaP NPs.

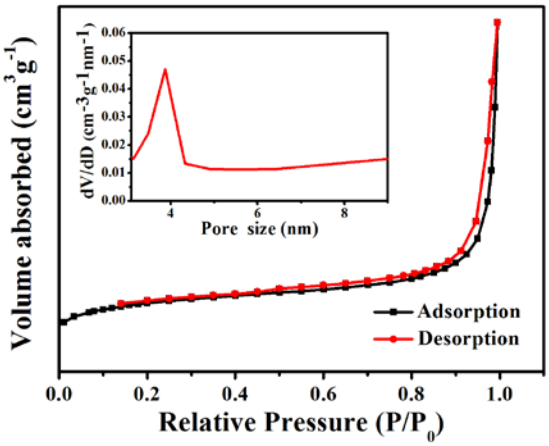

Figure S9. The N_2 adsorption/desorption isotherm and the pore size distribution curve (inset) of Au@C/CaP NPs.

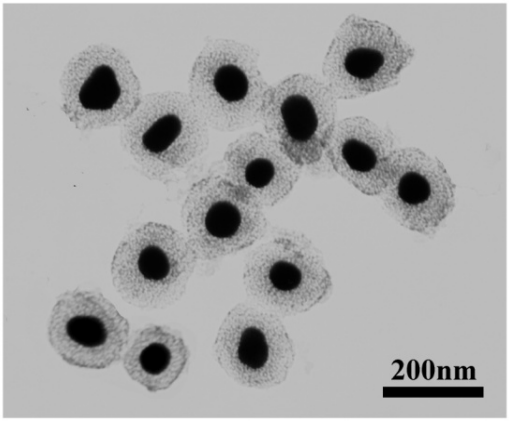

Figure S10. TEM image of Au@C core-shell NPs by dissolving Au@C/CaP NPs using the hydrochloric acid solution.

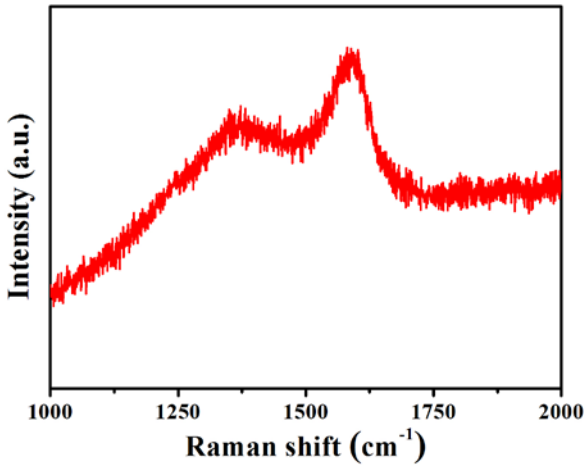

Figure S11. Raman spectrum of Au@C/CaP NPs.

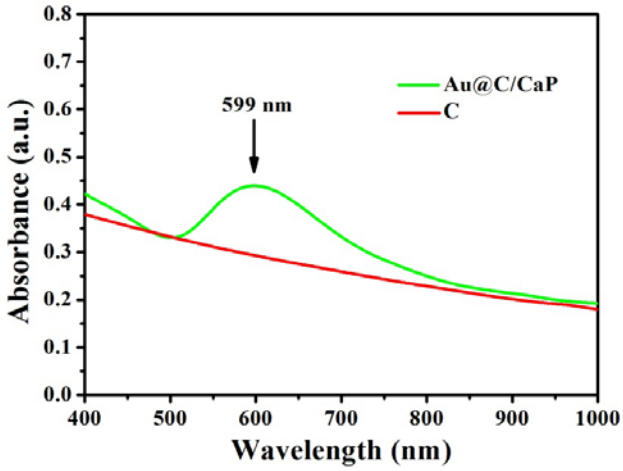

Figure S12. Vis-NIR absorbance of C (red) and Au@C/CaP NPs (green).

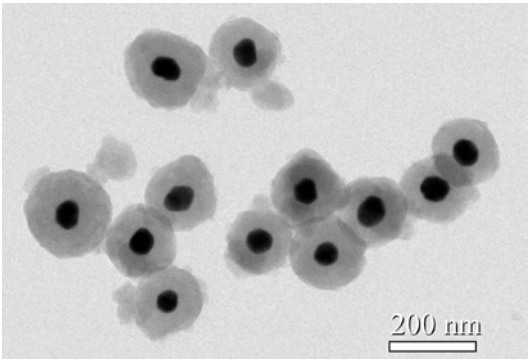

Figure S13. TEM image of Au@C/CaP NPs after four successive cycles of an on-and-off laser irradiation.

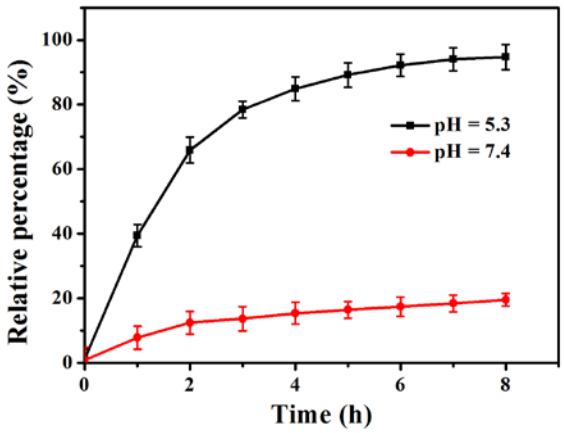

Figure S14. The Ca content profile for the Au@C/CaP NPs in the PBS (pH 5.3 and 7.4) in a certain time interval at 37 ºC.

1 N. G. Bastús, J. Comenge and V. Puntes, *Langmuir* 2011, **27**, 11098-11105.