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Electronic Supplementary Information (ESI)

Tunable fabrication of folic acid-Au@poly(acrylic acid)/mesoporous calcium phosphate Janus nanoparticles for CT imaging and active-targeted chemotherapy of cancer cells

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Experimental Section

Chemicals: Polyacrylic acid (PAA, $M_w \approx 1800$) and doxorubicin hydrochloride (DOX) were procured from Sigma (USA). Isopropyl alcohol (IPA), hydrogen tetrachloroaurate ($HAuCl_4 \cdot H_2O$), trisodium citrate, calcium hydroxide ($Ca(OH)_2$), sodium hydrogen phosphate (Na_2HPO_4) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Methoxy-poly(ethylene glycol)-thiol (OCH_3-PEG-SH) and folic acid (FA)-PEG-SH were obtained from Thermo Fisher Scientific. Deionized (DI) water was used in all experiments.

Characterization: Transmission electron micrographs (TEM) were recorded with a JEOL-2100F transmission electron microscope. Scanning electron microscopy (SEM) images and the energy dispersive X-ray (EDX) spectrum were carried out with a JEOL JSM-7610F scanning electron microscope. X-Ray photoelectron spectra (XPS) were measured on an ECSALAB 250 using monochromatic Al-Kα radiation. Fourier

transform infrared (FTIR) spectra were performed by a Magna 560 FTIR spectrometer. X-ray diffraction (XRD) patterns were measured by using a D8 Focus diffractometer (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). The size of the Janus nanoparticles (at a concentration of 0.05 mg mL^{-1}) were measured by dynamic light scattering (DLS) using a Malvern Zeta Sizer (Nano-ZS). Inductively coupled plasma atomicemission spectroscopy (ICP-AES) was measured with Leeman ICP-AES Prodigy. X-ray computed tomography (CT) images were obtained by using a SIEMENS SOMATOM Sensation 64, and the Hounsfield unit (HU) variations were determined by using a syngo CT 2009S instrument (Siemens, Berlin).

Synthesis of Au@PAA/mCaP JNPs with different ligand combinations

FA-Au@PAA/mCaP JNPs with FA-PEG-SH and PAA-Ca as ligands. Monodispersed Au NPs about 50 nm were prepared by reduction of tetrachloroauric acid by sodium citrate.[1] In a 100 mL flask, PAA (100 μL, 0.2 g mL^{-1}) and 8 mg of $Ca(OH)_2$ were mixed in 20 mL of DI water with magnetic stirring for 30 min. Then, 25 mL of as-synthesis Au NPs were added into the solution to form a suspension. To this mixture, 500 μL FA-PEG-SH (0.18 mg mL^{-1} in DI water) were added. 30 min later, 50 mL of IPA was dripped into the suspension under magnetic stirring to form the FA-Au@PAA-Ca JNPs. With continuous stirring for 5h, Na_2HPO_4 solution (24 mg of Na_2HPO_4 in 500 μL DI water) was added into the flask under magnetic stirring. The reaction mixture was reacted for 12 h under magnetic stirring to obtain the FA-Au@PAA/mCaP JNPs.

The eccentric FA-Au@PAA-Ca JNPs were synthesized using the same method, except that 250 μL FA-PEG-SH was used to replace 500 μL FA-PEG-SH. The content of the FA-PEG-SH was increased to 1500 μL. Similar synthesis led to unencapsulated Au NPs. A control experiment was carried out by using PAA-Ca. Concentric core-shell Au@PAA/mCaP NPs were obtained.

Au@PAA/mCaP JNPs with OCH_3-PEG-SH and PAA-Ca as ligands. The

synthesis is identical to that of Au@PAA/mCaP JNPs, except for the utilization of OCH_3-PEG-SH to replace FA-PEG-SH.

DOX loading and pH-responsive controlled release *in vitro*

1 mg of FA-Au@PAA/mCaP JNPs (1 mL of the obtained FA-Au@PAA/mCaP JNPs solution were collected by centrifugation, dried in air and weighed by using balance. Subsequently, the amount of 1 mg of JNPs was calculated and collected to load anti-cancer drug) and 100 μL of DOX solution (10 mg mL^{-1}) were mixed in 3 mL of DI water and stirred for 48 h at room temperature. DOX-loaded FA-Au@PAA/mCaP JNPs 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 FA-Au@PAA/mCaP JNPs *in vitro*, 2 mL of DOX-loaded JNPs was equally divided into two centrifuge tubes and centrifuged. Then, there were dispersed into 1 mL of phosphate buffered saline (PBS, pH 5.3 and 7.4) and kept releasing in water bath at 37 ℃. 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 mCaP. The FA-Au@PAA/mCaP JNPs (1 mg) were placed into pH 5.3 and 7.4 PBS buffer (4 mL), respectively. The supernatants were gathered at selected time intervals by centrifugation and tested by ICP-AES to measure the content of Ca.

Cell culture

The malignant lung cancer A549 cells and human cervical carcinoma cancer Hela cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (*v*/*v*) fetal bovine serum (Gibco) at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Cellular uptake

A549 and Hela cells (1×10^5) were cultured onto glass cover slips in a 24-well plate

for 24 h to allow the cells to attach, followed by treating with DOX-loaded Au@PAA/mCaP JNPs, DOX-loaded FA-Au@PAA/mCaP JNPs in normal culture medium and DOX-loaded FA-Au@PAA/mCaP JNPs in culture medium with free FA (5 mg mL^{-1}), respectively. The concentration of DOX in each group was 4 μg mL^{-1}. After 3 h incubation, cell imaging was conducted to evaluate the targeting efficiency of formulations to cells with CLSM. In addition, cell samples were collected and quantitatively analyzed by using FCM.

Cell cytotoxicity *in vitro*

The cell viability of empty FA-Au@PAA/mCaP JNPs, empty Au@PAA/mCaP JNPs, free DOX, DOX-loaded Au@PAA/mCaP JNPs and DOX-loaded FA-Au@PAA/mCaP JNPs were 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 ℃ for 24 h. Then, the medium was replaced by serum-free DMEM containing different concentrations of DOX-loaded FA-Au@PAA/mCaP JNPs. 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 ℃. 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):

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

CT imaging of FA-Au@PAA/mCaP JNPs *in vitro*

The CT images of Au@PAA/mCaP JNPs samples in PBS with various Au concentrations (0, 0.29, 1.16, 3.25, 5.23 and 7.52 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 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.

CT imaging of FA-Au@PAA/mCaP JNPs in Hela cells

1.0 × 10^5 mL^{-1} Hela cells were seeded in a 24-well plate in DMEM medium followed by incubation with FA-Au@PAA/mCaP JNPs with various Au concentrations (0, 0.18, 0.96, 2.73, 4.60 and 6.05 mg mL^{-1}) for 24 h at 37 ℃. After being washed with PBS three times the cells were suspended in 0.2 mL PBS for CT imaging.

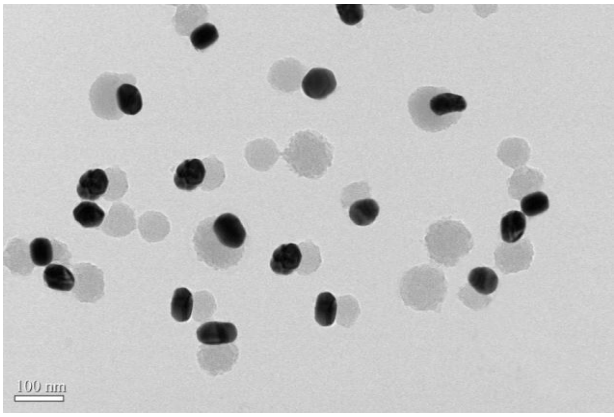

Fig. S1 A typical TEM image of the Au@PAA/mCaP JNPs prepared with using ligand OCH_3-PEG-SH.

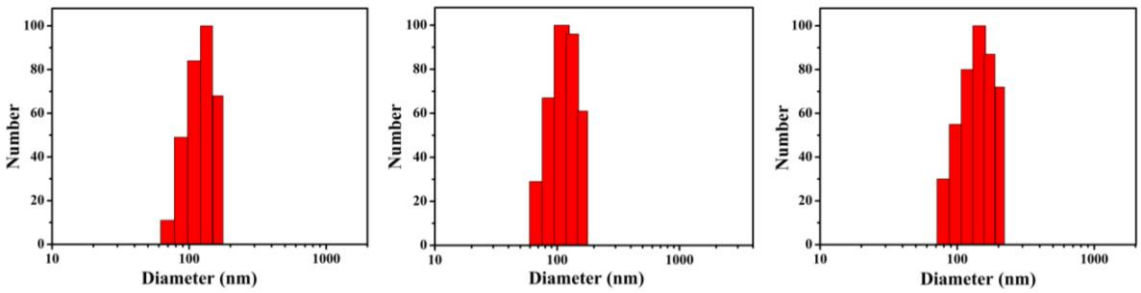

Fig. S2 Size distributions of the Au@PAA/mCaP JNPs dispersed in water (A), PBS (pH = 7.4) (B) and culture medium with serum (C).

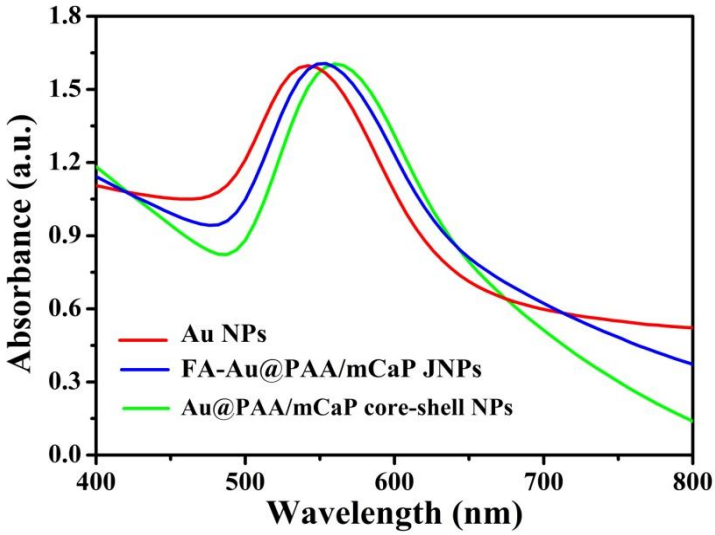

Fig. S3 UV-Vis absorption spectra of the as-synthesized AuNPs (red), FA-Au@PAA/mCaP JNPs (blue) and Au@PAA/mCaP core-shell NPs (green).

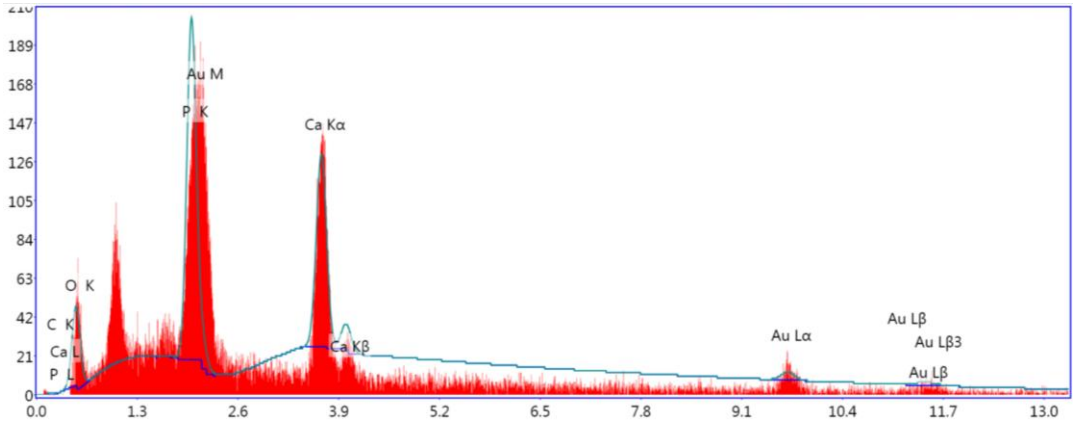

Fig. S4 EDX spectrum of FA-Au@PAA/mCaP JNPs.

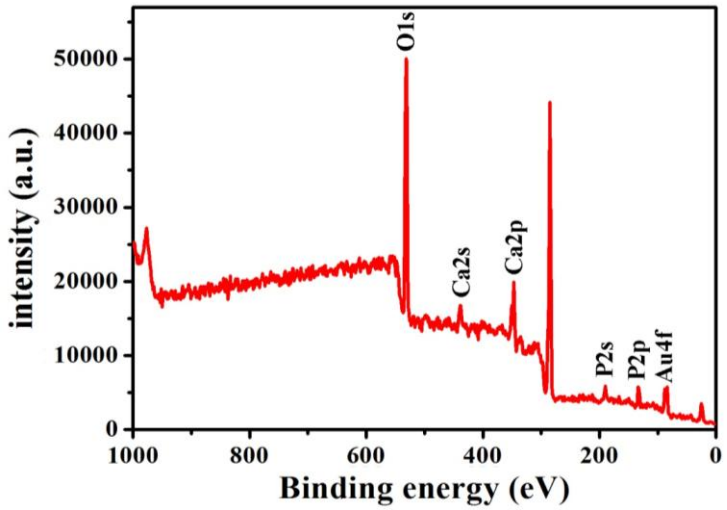

Fig. S5 XPS of FA-Au@PAA/mCaP JNPs.

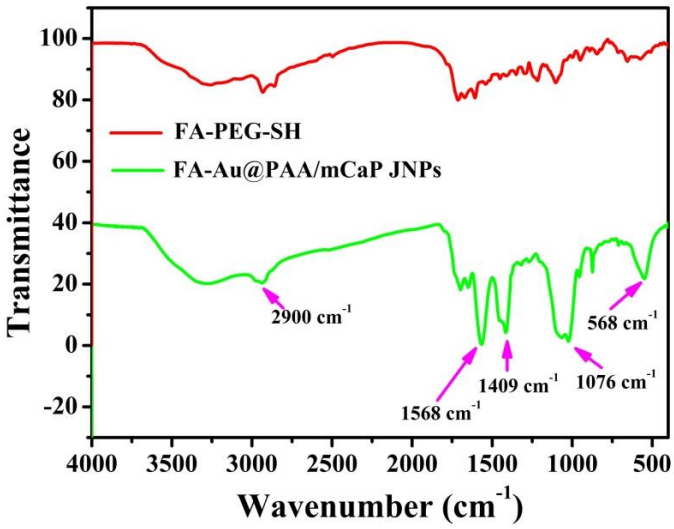

Fig. S6 FTIR spectra of FA-PEG-SH and FA-Au@PAA/mCaP JNPs.

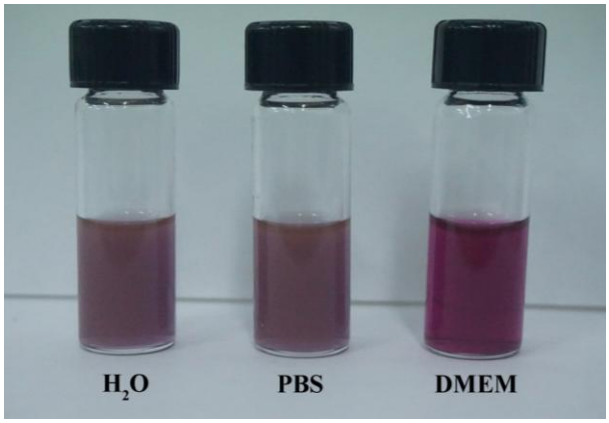

Fig. S7 Photographs of the FA-Au@PAA/mCaP JNPs in water, PBS buffer and culture medium with serum and they were stored for 36 h, respectively.

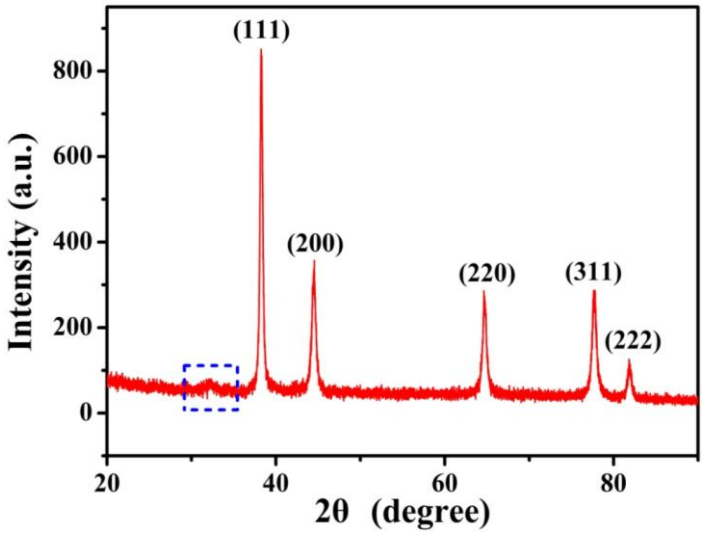

Fig. S8 XRD pattern of FA-Au@PAA/mCaP JNPs.

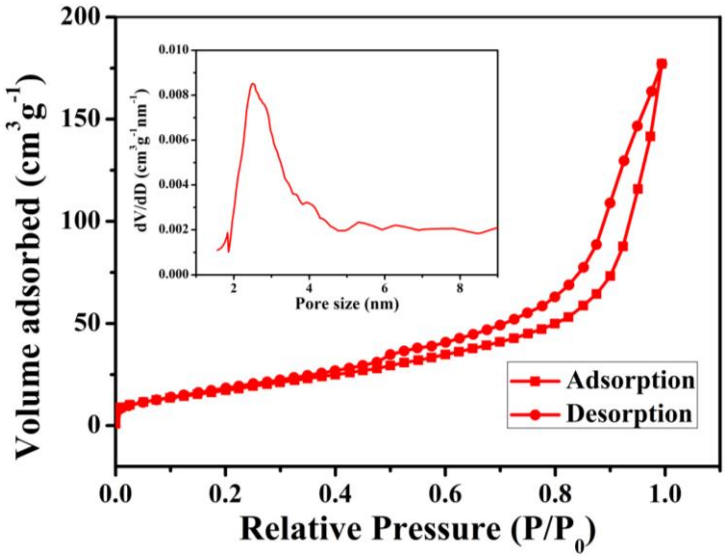

Fig. S9 The N_2 adsorption/desorption isotherm and the pore size distribution curve (inset) of FA-Au@PAA/mCaP JNPs.

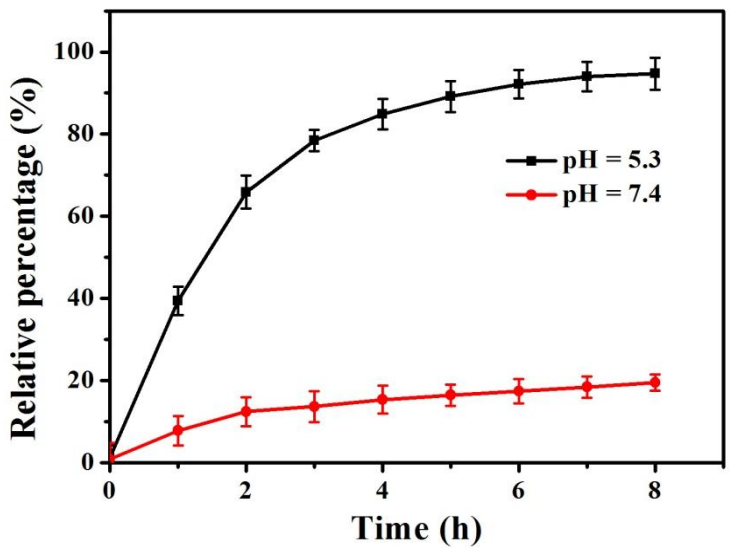

Fig. S10 The Ca content profile for the FA-Au@PAA/mCaP JNPs in the PBS (pH 5.3 and 7.4) in a certain time interval at 37 ℃.

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