Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

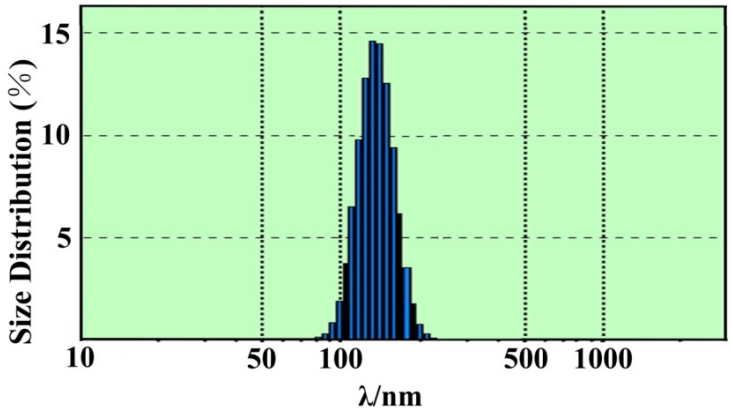

Figure S1. Size distribution of A-AuNCs@PAA/mSiO$_2$ NPs.

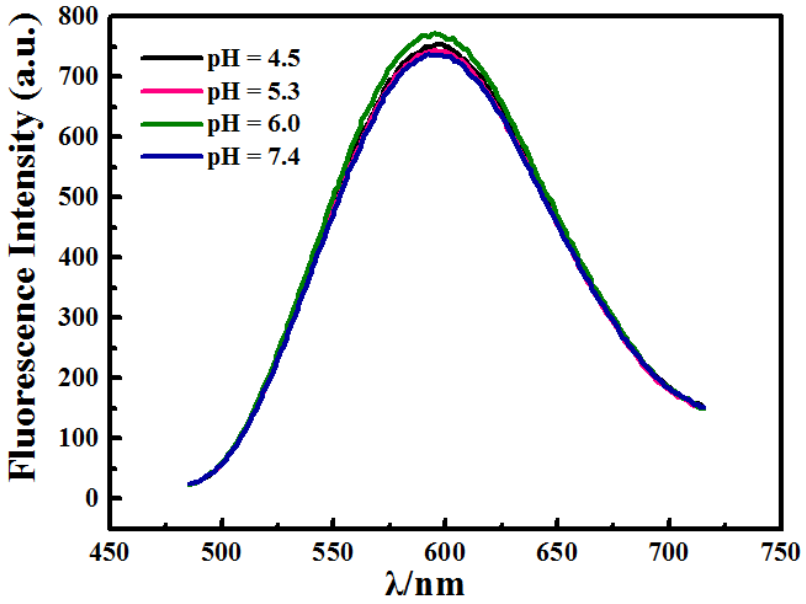

Figure S2. PL spectra (λ_{ex} = 365 nm) of A-AuNCs@PAA/mSiO$_2$ NPs in PBS buffer solutions of pH 7.4, 6.0, 5.3 and 4.5 at 37 °C, respectively.

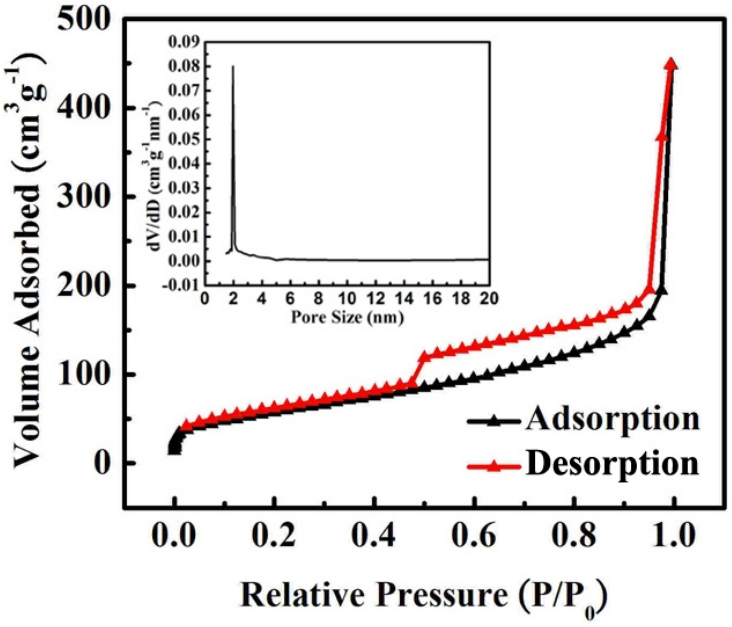

Figure S3. N_2 adsorption/desorption isotherm and pore size distribution curve (inset) of A-AuNCs@PAA/mSiO_2 NPs.

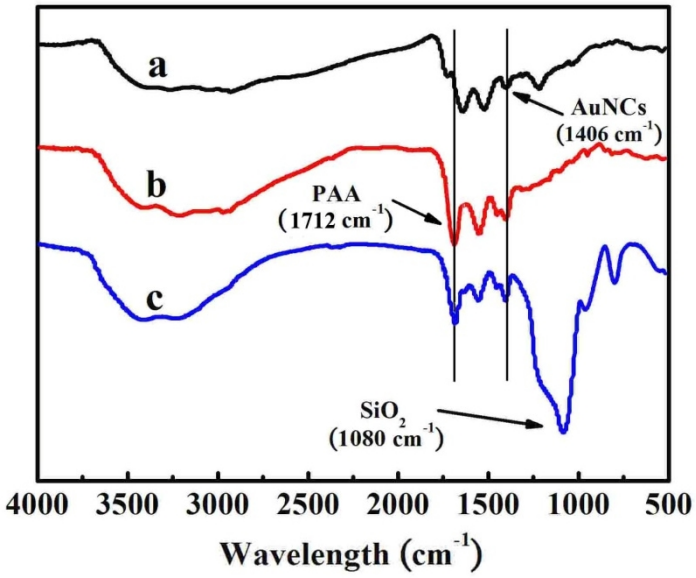

Figure S4. FTIR spectra of (a) AuNCs, (b) A-AuNCs@PAA NPs and (c) A-AuNCs@PAA/mSiO_2 NPs.

The peaks at 1406 cm^{-1} that appear in three characteristic curves are assigned to the unique vibration mode of GSH. The obvious peak at 1712 cm^{-1} in curve b and c are attributed to the C=O stretching vibration in the carboxyl group, qualitatively indicating that the PAA polymer existed in A-AuNCs@PAA NPs and A-AuNCs@PAA/mSiO_2 NPs. In curve c, the

absorption at 1080 cm^{-1} results from Si-O-Si antisymmetric stretching vibration, which implies the existence of a SiO_2 layer on the A-AuNCs@PAA NPs surface. These results confirm that PAA has successfully assembled onto A-AuNCs and then encapsulated in $mSiO_2$ nano-composites shell, eventually forming the A-AuNCs@PAA/$mSiO_2$ NPs.

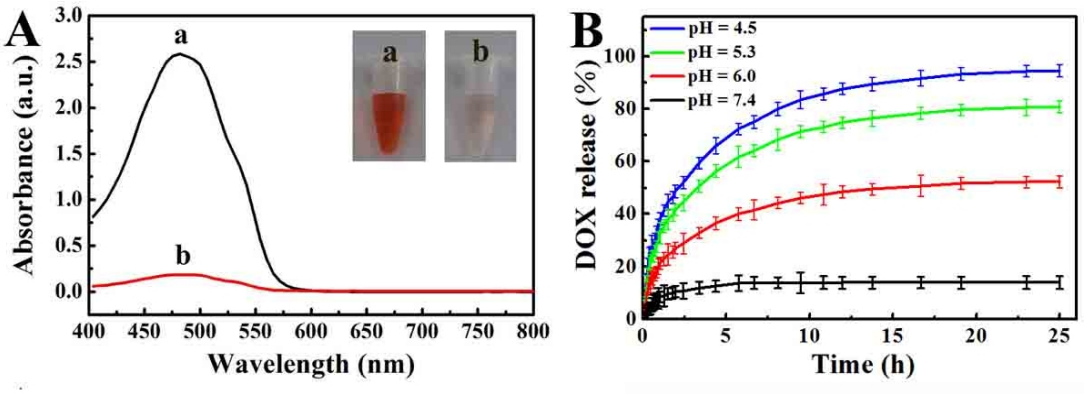

Figure S5. (A) UV–Vis absorption spectra of DOX solutions before (a) and after (b) interacting with A-AuNCs@PAA/$mSiO_2$ NPs. Inset: photographs of (a) the mixture of A-AuNCs@PAA/$mSiO_2$ NPs and DOX, (b) supernatant after centrifugation of (a). (B) DOX-release profiles for DOX-loaded A-AuNCs@PAA/$mSiO_2$ NPs measured in PBS buffer solutions of pH 7.4, 6.0, 5.3 and 4.5 at 37 °C, respectively.

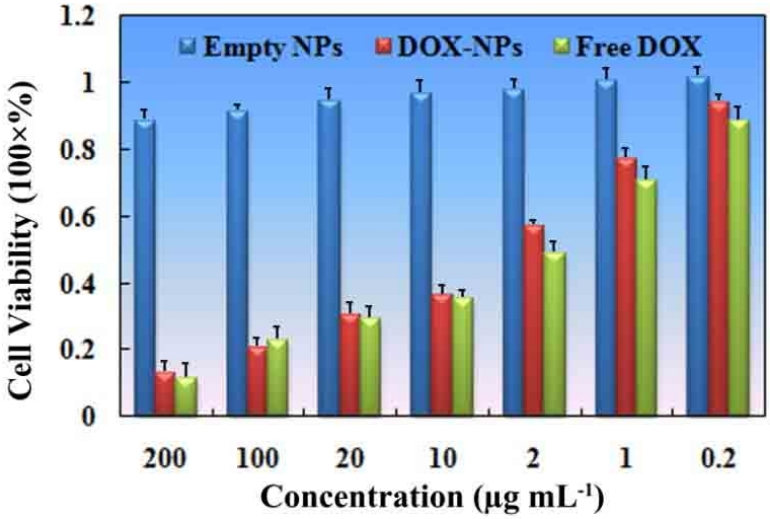

Figure S6. *In vitro* cytotoxicity studies of A-AuNCs@PAA/$mSiO_2$ NPs, DOX-loaded A-AuNCs@PAA/$mSiO_2$ NPs and free DOX against HepG-2 cells at different levels of concentration after 24 h.

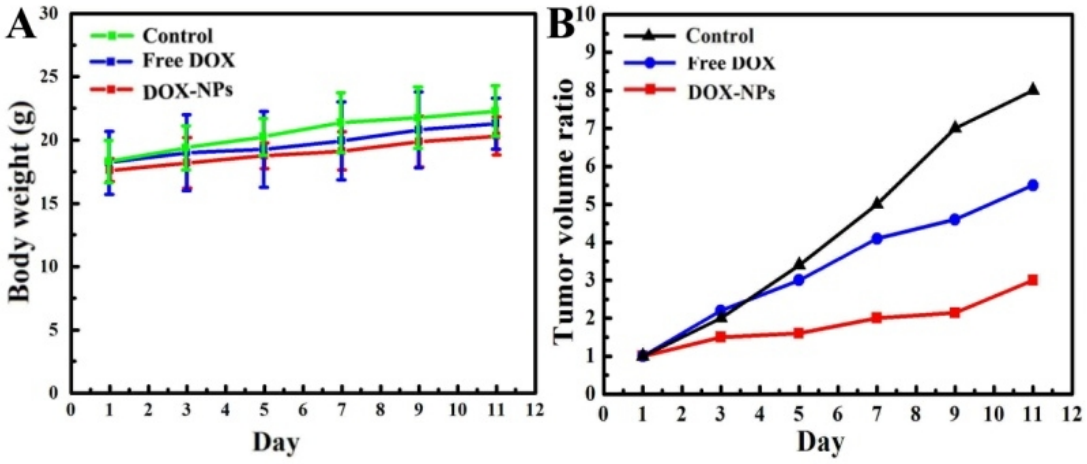

Figure S7. (A) Body weight and (B) relative tumor volume recorded for mice after treatment with saline solution as a control, free DOX and DOX-loaded A-AuNCs@PAA/mSiO_2 NPs.

Chemicals.

Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$), tetraethyl orthosilicate (TEOS, ≥98%), polyacrylic acid (PAA, $M_w \approx 800$) and doxorubicin hydrochloride (DOX) were purchased from Sigma (USA). Glutathione (GSH) was purchased from Beijing Dingguo Biotechnology Co., Ltd. Isopropanol and ammonia solution (25-28%) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Deionized water (DI-water) was used in all experiments.

Characterization.

Transmission electron microscopy (TEM) was performed on a JEOL-100CX transmission electron microscope at 80 kV (Hitachi, Japan). The high-resolution TEM (HR-TEM) characterizations were performed with a TECNAI G2 F20 transmission electron microscope under 200 kV accelerating voltage. Photoluminescence (PL) spectra were performed with Eclipse fluorescence spectrophotometer (Varian, USA). Scanning electron microscopy image (SEM) was obtained using an XL30 ESEM-FEG field-emission scanning electron microscope (FEI Co.). N_2 adsorption/desorption measurements were performed by an intelligent gravimetric analyzer Autosorb-iQ (Quantachrome). Confocal laser scanning microscopy (CLSM) was operated on Olympus Fluoview FV1000. UV-vis absorption spectra were obtained on U-3010 spectrophotometer (Hitachi, Japan). Inductively coupled plasma atomic

emission spectroscopy (ICP-AES) was measured with Leeman ICP-AES Prodigy. Fourier transform infrared spectroscopy (FTIR) spectra were obtained on a Magna 560 FTIR spectrometer (Nicolet, USA). Particle size distribution was obtained using a Mastersizer 2000 laser particle size analyzer. X-ray computed tomography (CT) images were obtained using a SIEMENS SOMATOM Sensation 64, and the Hounseld Unit (HU) variations were acquired and analyzed using syngo CT 2009S (Siemens, Berlin, Germany).

Synthesis of glutathione capped gold nanoclusters (GSH-AuNCs).

According to the previously reported method, AuNCs were synthesized through reduction of $HAuCl_4$ with GSH.[1] Briefly, 20 mM $HAuCl_4$ (0.5 mL) was added to DI-water (4.35 mL) followed by the addition of GSH solution that was prepared by adding 0.0046 g GSH into DI-water (0.15 mL). The mixture was gently stirred at 25 ^oC for 5 min, and then the reaction mixture was heated to 70 ^oC under gentle stirring for 24 h. At last, orange-emitting AuNCs were achieved.

Synthesis of A-AuNCs@PAA NPs.

To obtain A-AuNCs, 500 μL as-prepared AuNCs and 2 mL isopropanol were mixed under vigorously stirring for 5 min. Then, 2 mL H_2O and 7 μL of PAA aqueous solution (0.2 g mL^{-1}) were added, and the suspension was vigorously stirred for 30 min to disperse PAA sufficiently. Afterwards, 10.5 μL $NH_3 \cdot H_2O$ (2 M) were added into the above solution and stirred for another 30 min. Finally, 8 mL of isopropanol were added drop by drop into the mixture under magnetic stirring to obtain A-AuNCs@PAA NPs.

Synthesis of A-AuNCs@PAA/mSiO_2 NPs.

After adding 12 mL of isopropanol into 3 mL of the as-prepared A-AuNCs@PAA NPs solution, we adjusted the pH value to 7-8 with $NH_3 \cdot H_2O$ solution (2 mol L^{-1}). Then, the addition of 500 μL of TEOS (20% in isopropanol) was carried out ten times for every 30 min intervals. After stirring for 24 h, the resulting A-AuNCs@PAA/mSiO_2 NPs were collected by centrifugation and washed with isopropanol and DI-water repeatedly so that the excess precursors were removed.

CT imaging in PBS (pH=7.4).

The CT images of A-AuNCs@PAA/mSiO$_2$ NPs samples in phosphate buffer solution (PBS) with various NPs concentrations (0, 60, 120, 180, 240, 300 and 360 mg mL^{-1}) were obtained using a SIEMENS SOMATOM Sensation 64 with a tube voltage of 120 kV, an electrical current of 280 mA, a slice thickness of 1.0 mm. Phantom images were treated using a standard image viewer application to measure the mean HU variation of the acquired image depending on different concentrations of A-AuNCs@PAA/mSiO$_2$ NPs.

Cell Culture.

Human hapetocelluar carcinoma (HepG-2) cells were maintained in a humidified incubator in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum and cultured at 37 °C in an atmosphere of 95% air/5% CO_2.

Cell Uptake.

In a typical procedure, 1.0 × 10^5 mL^{-1} HepG-2 cells were seeded onto glass cover slips in a 24-well plate in IMDM medium containing 10% fetal bovine serum for 24 h at 37 ^{o}C in a humidified atmosphere with 5% CO_2 to allow the cells to attach. Then the medium was replaced with 400 μL culture serum-free medium containing A-AuNCs@PAA/mSiO$_2$ NPs (25 μg mL^{-1}). After incubation for 24 h, the cell monolayer on the glass sheet was washed with PBS (pH=7.4) for three times to remove the remaining A-AuNCs@PAA/mSiO$_2$ NPs and dead cells, and then sealed with a microscope glass slide. Finally, the observations were performed by using CLSM.

Loading DOX into A-AuNCs@PAA/mSiO$_2$ NPs.

UV–Vis spectroscopy was used to determine the amount of DOX loaded into the NPs. A stock solution of DOX (2 mg mL^{-1}) was used as a standard and serially diluted to concentrations of 4-100 μg mL^{-1} in deionized water. The serial DOX solutions of different concentration were measured at 480 nm and a linear fit of the data was created and used as standard curve for the absorption against DOX concentration. The drug-loaded NPs were prepared by mixing DOX aqueous solution (10 mg mL^{-1}, 60 μL) with A-AuNCs@PAA/mSiO$_2$ NPs (1.0 mg mL^{-1}, 1.0 mL) for 48 h and then centrifuged at 9000 rpm for 10 min. To evaluate the DOX-loading efficiency, the contents of original DOX and residual DOX

in supernatant were determined by UV–Vis measurements at 480 nm and compared to the standard curve created previously. The loading efficiency (LE%) of DOX is calculated by using equation 1:

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

DOX release behaviors of DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs.

In vitro release of DOX from DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs was evaluated using a semi-permeable dialysis bag diffusion technique right after the DOX loading. The as-prepared DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs were redispersed in 0.5 mL PBS (pH = 7.4, 6.0, 5.3 and 4.5). All of the release mediums were placed into pre-treated semi-permeable dialysis bags and then immersed into 6 mL corresponding PBS solutions (pH = 7.4, 6.0, 5.3 and 4.5) at 37 ^{o}C with gentle shaking. At certain time intervals, DOX concentration moved out of semi-permeable dialysis dag into water was measured by fluorescence spectrophotometer. The amount of DOX released was determined by fluorescence emission at 591 nm with excitation at 480 nm.

In vitro cytotoxicity against HepG-2 cells.

The *in vitro* cytotoxicity of empty A-AuNCs@PAA/mSiO$_2$ NPs, DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs and free DOX were evaluated by standard 3-(4, 5-dimethylthialzol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays and HepG-2 cells were used. Cells were seeded in a 96-well plate at a density of 2.5×10^4 per well and incubated at 37 ^{o}C in a humidified atmosphere with 5% CO_2 in IMDM medium containing 10% fetal bovine serum for 24 h to attach. Then serial concentrations of empty A-AuNCs@PAA/mSiO$_2$ NPs, DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs and free DOX in serum-free medium with 100 μL were added, respectively. One row of a 96-well plate was used as a control with 100 μL culture medium only. After 24 h incubation, 20 μL 5 mg mL^{-1} MTT solution was added to each well and the mixture was incubated for another 4 h. The amount of dark-blue formazan crystals generated by the live cells was proportional to the number of live cells. The medium was then replaced with DMSO (150 μL) and the absorbance was monitored with a microplate reader at a wavelength of 490 nm. Cell viability was determined by equation 2:

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

Animal.

All animal experiments were performed in accordance with the principles and procedures described in ‘‘Regulations for the Administration of Affairs Concerning Laboratory Animals’’ and ‘‘The National Regulation of China for Care and Use of Laboratory Animals’’. Balb/c female mice (18-22 g) were purchased from Center for Experimental Animals, Jilin University. Animals were housed under normal conditions with 12 h light and dark cycles and given access to food and water ad libitum. All animal experiments were in agreement with the guidelines of The National Regulation of China for Care and Use of Laboratory Animals.

Fluorescence and CT imaging of A-AuNCs@PAA/mSiO$_2$ NPs *in vivo*.

For *in vivo* imaging, A-AuNCs@PAA/mSiO$_2$ NPs were dispersed in PBS buffer solution at the concentration of 100 mg mL^{-1}. Afterwards, 200 μL of the as-prepared A-AuNCs@PAA/mSiO$_2$ NPs was in situ injected into the tumor of the narcotic mouse (anesthetized with 0.7% sodium pentobarbital at 10 mL per kg). Then fluorescent and CT images of A-AuNCs@PAA/mSiO$_2$ NPs *in vivo* were achieved successively under the following conditions: (a) *In vivo* fluorescence imaging was performed by using a CRIMaestroTM *in vivo* imaging system with more than 515 nm emission filter and 445-490 nm excitation filter. (b) The CT imaging parameters were setted as follows: thickness, 1.0 mm; pitch, 120 kV, 280 mA; field of view, 300 mm; gantry rotation time, 4.95 s.

Anti-tumor growth assay.

The liver cancer cell lines (H-22) bearing Balb/c mice were randomly divided into three groups with five animals in each group, which were treated by tail vein injection of DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs, the same dose of pure DOX and saline were used as a control. On each treatment day, saline solution (0.9%, 0.2 mL), DOX (0.3 mg mL^{-1}, 0.2 mL), and DOX-loaded A-AuNCs@PAA/mSiO$_2$ NPs (0.54 mg mL^{-1}, 0.2 mL, and the concentration of DOX was 0.3 mg mL^{-1}) were intravenously injected, respectively. The body weights and tumor volumes were monitored every other day after treatment. The length of the major (longest diameter) and minor (perpendicular to the major axis) axes of the tumor were measured with a vernier caliper, and the tumor volume was calculated as described previously. After treatment for 11 days, animals from each group were randomly chosen and euthanized to

retrieve tumors. The excised tumors were washed with deionized water and fixed with 4% (weight) paraformaldehyde solution. Tumor inhibitory rate was determined by equation 3:

Tumor inhibitory rate (%) = (Tumor weight$_{(control\ group)}$-Tumor weight$_{(treat\ group)}$)/Tumor weight $_{(control\ group)}$×100%

1 Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, *J. Am. Chem. Soc.*, 2012, **134**, 16662.