

## SUPPORTING INFORMATION

### **Enzyme-Triggered Self-Assembly of Gold Nanoparticles for Enhanced Retention Effect and Photothermal Therapy of Prostate Cancer**

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## 1. General information

Unless otherwise stated, all organic solvents were dried and distilled before use. Water was purified by the Millipore filtration system. All reagents and chemicals were in AR grade and were used without further purification unless otherwise noted. Reactions were performed under an argon atmosphere unless otherwise specified. Anhydrous dichloromethane (DCM) and dimethyl formamide (DMF) were distilled over  $\text{CaCl}_2$  and  $\text{CaH}_2$  respectively and kept anhydrous with 4Å molecular sieves. N, N-diisopropylcarbodiimide (DIC), N-Hydroxybenzotriazole (HOBt) and Fmoc-protected amino acid were purchased from GL Biochem (Shanghai, China) Ltd. Recombinant intestinal alkaline phosphatase (ALP) was obtained from BaoMan Inc. (Shanghai, China) (one unit (U) is the enzyme activity that cleaves 1  $\mu\text{mol}$  of the standard substrate per minute at 37 °C). The phosphatase inhibitor complex II was bought from Sangong Biotech Inc. (Shanghai, China).

The mass spectra of peptides were measured with an AB Sciex (MALDI-TOF) mass spectrometer. The High-Performance Liquid Chromatography (HPLC) analysis was performed with the following parameters: RP-C18 HPLC column (10  $\mu\text{m}$  particle size) and UV detector. The mobile phase was a gradient of 10%-90% of methanol aqueous solution containing 0.5% trifluoroacetic acid at a total flow rate of 16 mL/min. The pH was measured by a Mettler Toledo FE 20 K pH meter.

## 2. General procedure for synthesis of peptide

### Fmoc removal

The Fmoc protecting group was cleavage by treatment with 20% piperidine in DMF (2×20 mL, 5 min each) under microwave radio condition (35 W, 60±5 °C, 5 min). Then, the resin was washed with DMF (7×10 mL, *ca.* 1 min each) to remove the last traces of piperidine. A positive result of the Kaiser test confirmed the cleavage of the Fmoc group and the presence of free amino group.

### Standard Fmoc solid-phase peptide synthesis techniques (SPPS)

Each amino acid was attached using 0.81 mmol/g loading Fmoc Rink amide resin under microwave radio (35 W, 60±5 °C, 20 min). Then, the resin was washed 7×10 mL with DMF (*ca.* 1 min each) to remove the last traces of the amino acid. A negative result of the Kaiser test confirmed the attachment of the corresponding amino acid.

### Cleavage from the Resin

Cleavage of the product from the resin was achieved by treatment with a mixture solution of TFA-H<sub>2</sub>O-triisopropylsilane (95:2.5:2.5) for 2 h. The cleavage mixture was collected by filtration and the resin was washed twice with pure TFA (10 mL). The filtrates were combined and concentrated under vacuum to obtain oily residue. The peptide was precipitated by adding dry cold diethyl ether to the oil, followed by centrifugation of the mixture. To obtain the blue hydrochloride salt, the solid was dissolved in water (10 mL), acidified with hydrochloric acid (10%, 1 mL) and lyophilized. This step was repeated three times. Purity of the peptides was checked by HPLC on a RP-C18 column using water/MeOH as eluent.

### As starting Fmoc-based amino acids were used as followed

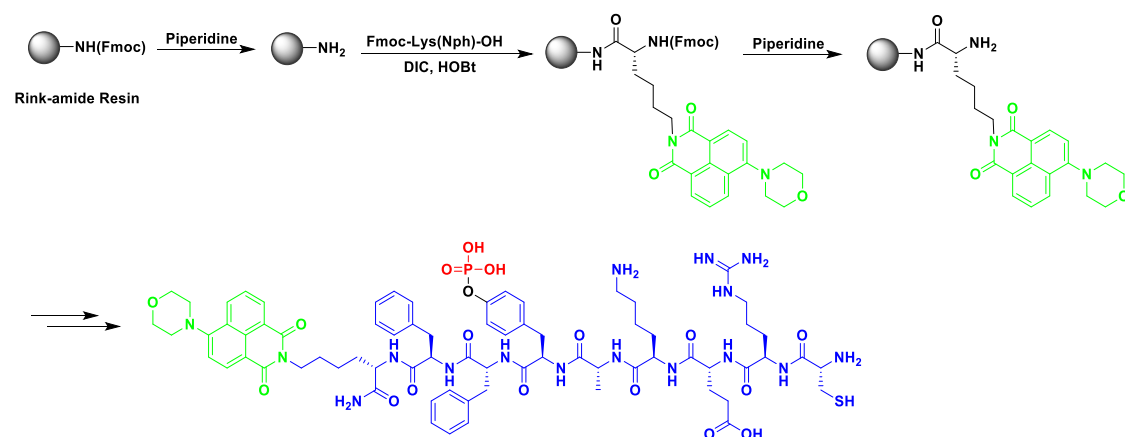
Fmoc-Lys(Nph)-OH; Fmoc-Phe-OH; Fmoc-Tyr(PO(OBzl)OH)-OH; Fmoc-Lys(Boc)-OH; Fmoc-Ala-OH; Fmoc-Glu(OtBu)-OH; Fmoc-Arg(Pbf)-OH; Fmoc-Cys(Trt)-OH.

### Synthesis of peptide CREKA-Y<sub>P</sub>FFK(Nph)

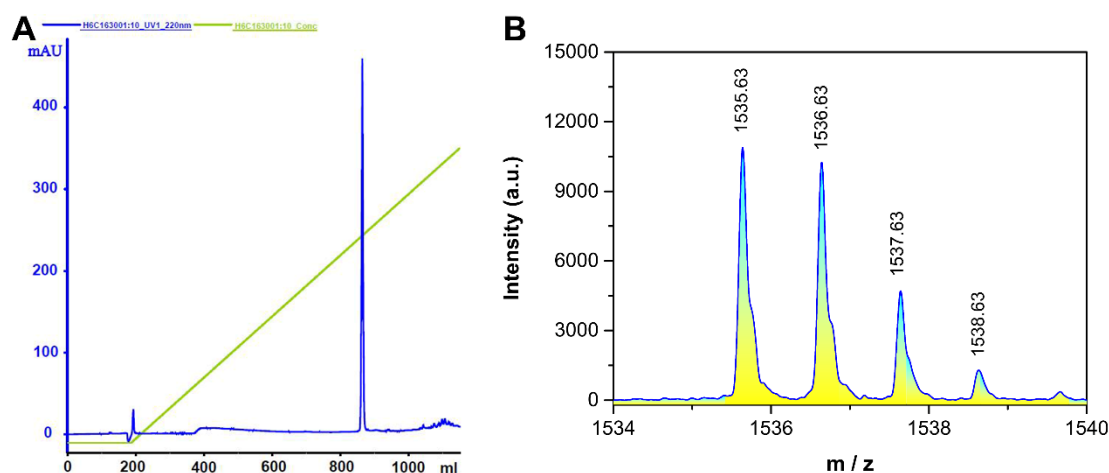
Rink amide resin (309 mg, 0.81 mmol/g, 0.25 mmol, 1 equiv.) was weighed out into a glass peptide synthesis vessel and allowed to swell in DMF (15 mL) for 2 h. Then, the Fmoc protection group was removed by treatment with piperidine (20%) in DMF (20 mL). After an intensive washing cycle with DMF, the following Fmoc-Lys(Nph)-OH and other amino acids were attached under microwave condition for SPPS: Fmoc-Lys(Nph)-OH (0.75 mmol, 3.0 equiv), DIC (2.5 mmol, 10.0 equiv), HOBt (0.75 mmol, 3.0 equiv); Fmoc-protected amino acid (0.75 mmol, 3.0 equiv), DIC (2.5 mmol, 10.0 equiv), HOBt (0.75 mmol, 3.0 equiv) in DMF (10 mL). The final product was cleaved from the solid support according to the general procedure for the Rink amide resin.

### Peptide CREKA-Y<sub>P</sub>FFK(Nph)

A green solid (40 mg, 0.026 mmol, Yield: 10.45%, purity HPLC: 99.0%), Mp: >300 °C. MALDI-TOF (m/z): calcd for 1534.65, found [M+H]<sup>+</sup> 1535.63.



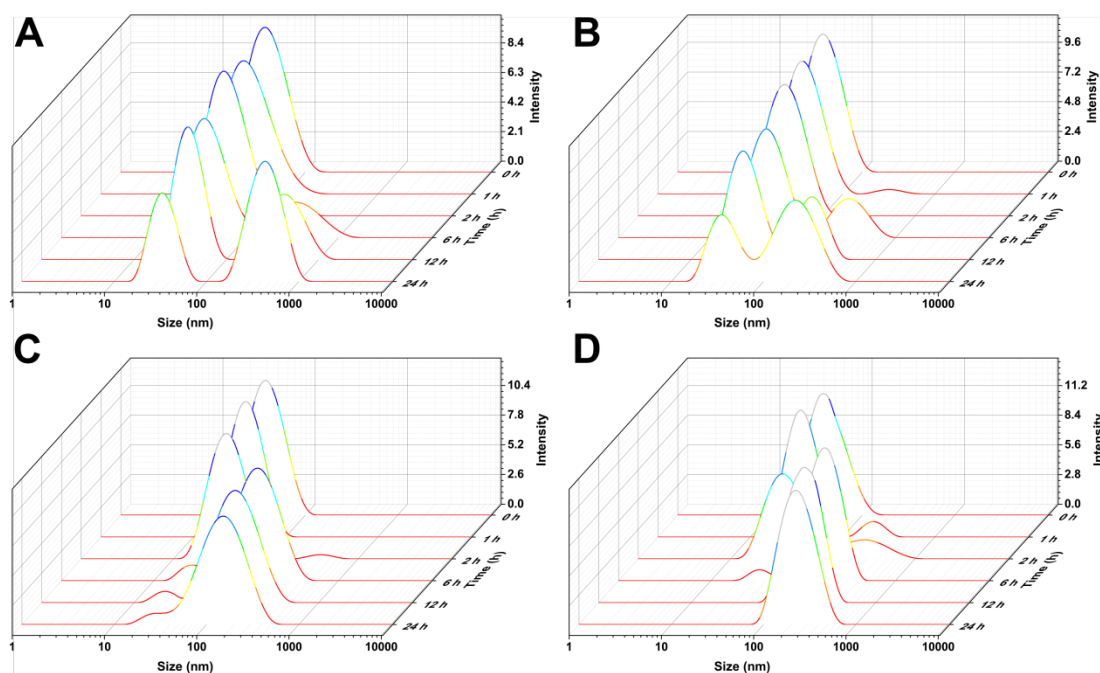
**Fig. S1** Synthesis of peptide CREKA-Y<sub>p</sub>FFK(Nph) with SPPS method.



**Fig. S2** (A) HPLC and (B) MALDI-TOF MS analysis of peptide CREKA-Y<sub>p</sub>FFK(Nph).

### 3. Dynamic light scattering (DLS)

The DLS experiments were determined by Nano-ZS (Zetasizer, Malvern) instrument, and starting solutions were filtered prior to use. Samples of AuNPs@Peptide with different ratio of AuNPs and peptide were all dissolved in PBS at pH 7.4 in a total sample volume of 1 mL, respectively. The ratio of AuNPs and peptide was 1:1250, 1:2500, 1:5000 and 1:10000, respectively.



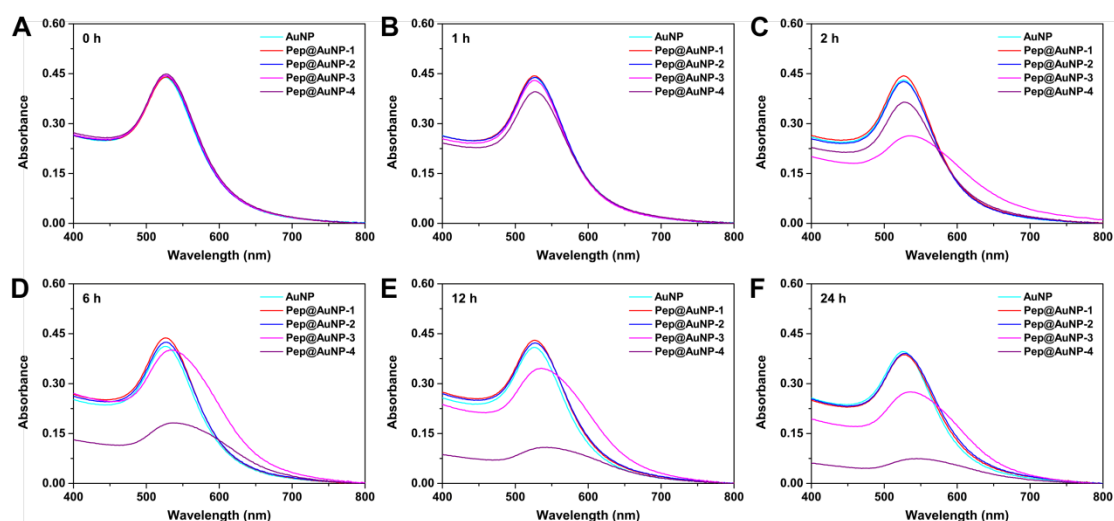
**Fig. S3** (A-D) Hydrodynamic size profiles of AuNPs@Peptide with different ratio of AuNPs and peptide before and after addition of ALP. (A) 1:1250; (B) 1:2500; (C) 1:5000; (D) 1:10000.

## 4. Transmission electron microscopy (TEM)

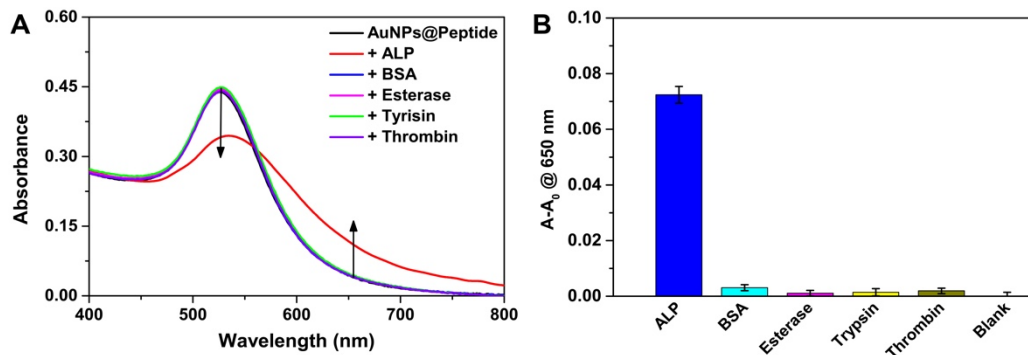
High-resolution images of AuNPs@Peptide were acquired using TEM. Samples were prepared respectively, by placing a few droplets of 50  $\mu\text{g/mL}$  AuNPs@Peptide in PBS (100 mM Tris, pH 7.4, 25  $^{\circ}\text{C}$ ) onto a carbon-coated grid with holes and dried at room temperature. TEM characterization was performed using a JEM-2100 electron microscope (JEOL, Japan).

## 5. UV/Vis and fluorescence spectrum experiments

Absorption spectra were recorded using a UV-visible spectrometer and fluorescence spectra were measured with fluorescence spectrometer (PerkinElmer LS55) at 25  $^{\circ}\text{C}$ . All spectra were corrected for intensity using the manufacturer-supplied correction factors and corrected for background absorption by subtracting a blank scan of the buffer system. AuNPs@Peptide synthesized with different ratio of AuNPs and peptide were all dissolved in PBS at pH 7.4.

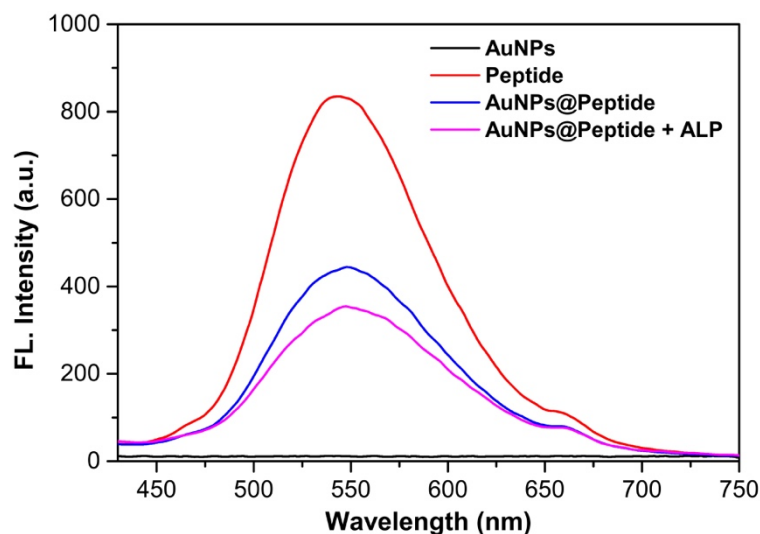


**Fig. S4** (A-F) Absorption spectra of AuNPs@Peptide with different ratio of AuNPs and peptide before and after addition of ALP. Pep@AuNP-1: the ratio was 1:1250; Pep@AuNP-2: the ratio was 1:2500; Pep@AuNP-3: the ratio was 1:5000; Pep@AuNP-4: the ratio was 1:10000.



**Fig. S5** (A) Absorption spectra of AuNPs@Peptide after addition of ALP or potential interfering

species. (B) Absorption increase of AuNPs@Peptide at 650 nm after addition of ALP or potential interfering species.



**Fig. S6** Fluorescence spectra ( $\lambda_{\text{ex}}$  405 nm) of AuNPs, Peptide, AuNPs@Peptide with or without ALP.

## 6. Cell experiments

### Cell culture

The human prostate cancer cell line PC-3 and human breast cancer cell line MCF-7 were purchased from Chinese Academy of Sciences Committee Type Culture Collection Cell Bank (Shanghai, China). PC-3 was routinely maintained in RPMI 1640 medium (Gibco) and MCF-7 was cultured in DMEM (Gibco) medium. All medium was supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (Gibco). Periodic testing ensured the absence of mycoplasma contamination. Cells were passaged using 0.25% Trypsin/EDTA (Gibco) when they reached 80%-90% confluence and seeded for the experiments.

### Confocal microscopic imaging

PC-3 cells and MCF-7 cells were seeded in a 35 mm petri dish with a glass cover slide and allowed to adhere overnight before treatment. Then the cells were incubated with 50  $\mu\text{g/mL}$  AuNPs@Peptide in complete medium for 24 h at 37  $^{\circ}\text{C}$ . After incubation, cells imaging was carried out after washing with PBS twice. Cell fluorescence images were obtained with a confocal laser scanning microscope (Nikon A1, Japan, 60 $\times$ oil-immersion objective lens). Channel 1 for AuNPs@Peptide, excitation: 405 nm, emission collected: 450-550 nm.

### Cell cytotoxicity assay

Cell viability was detected with the CCK-8 assay (Beyotime). The PC-3 cells and MCF-7 cells were plated in 96-well plates at a density of  $\sim 10^5$  cells/mL, with five duplicate wells of each group. The cells only incubation with AuNPs@Peptide were denoted as AuNPs@Peptide group, while cells receiving 650 nm irradiation in absence of AuNPs@Peptide were denoted as laser group, cells receiving 650 nm laser irradiation after incubation with AuNPs@Peptide were denoted as AuNPs@Peptide + laser group. After cultured for 24 h and washed with PBS twice, the cells were cultured with AuNPs@Peptide at the concentrations of 8  $\mu\text{g/mL}$ , 16  $\mu\text{g/mL}$ , 32  $\mu\text{g/mL}$ , 64  $\mu\text{g/mL}$ , 128  $\mu\text{g/mL}$  and 256  $\mu\text{g/mL}$  for 24 h, respectively. The exposure time for 650 nm laser ( $5 \text{ W/cm}^2$ ) was of 10 min. After the culture medium was extracted and the cells were washed with PBS twice, 10  $\mu\text{L}$  of CCK-8 solution was added to each well and incubated at  $37^\circ\text{C}$  for 4 h. The optical density was read at 450 nm using a spectrophotometer (BioTek). The following formula was used to calculate the viability of cell growth:  $\text{Viability (\%)} = (\text{mean absorbance value of the treatment group-blank} / \text{mean absorbance value of the control-blank}) \times 100$ .

### **Alkaline phosphatase assay kit**

The ALP activity was detected using alkaline phosphatase assay kit (Jiancheng) according to the manufacturer's instructions.

### **RNA isolation**

Total RNA was extracted from cultured cells with Trizol reagent (TaKaRa), according to the manufacturer's protocol. Quality and concentration of the RNA was assessed by measuring the ratios of absorbance using the NanoDrop 2000c Spectrophotometer (Thermo Scientific). A260/A280 and A260/A230 ratios  $> 1.8$  were considered suitable for further analysis.

### **Real-time qRT-PCR**

For quantitative reverse transcription–polymerase chain reaction (qRT-PCR) of mRNA, 1  $\mu\text{g}$  of DEPC-dissolved RNA was reversed transcribed using PrimeScript™ RT Master Mix (TaKaRa). The cDNA was appropriately diluted and was used in a qRT-PCR reaction with TB Green™ Premix Ex Taq™ II (TaKaRa). The relative gene expression levels were calculated using  $2^{-\Delta\Delta C_t}$  analysis method. GAPDH was used as the internal control.

Fibronectin PCR primers are listed as follow:

Fibronectin F: 5'-CGTCTCTCCCCACCGTCT-3'

Fibronectin R: 5'-GGTCCGCTCCCACTGTTGATT-3'

ALPL PCR primers are listed as follow:

ALPL F: 5'-ACGTGGCTAAGAATGTCATC-3'

ALPL R: 5'-CTGGTAGGCGATGTCCTTA-3'



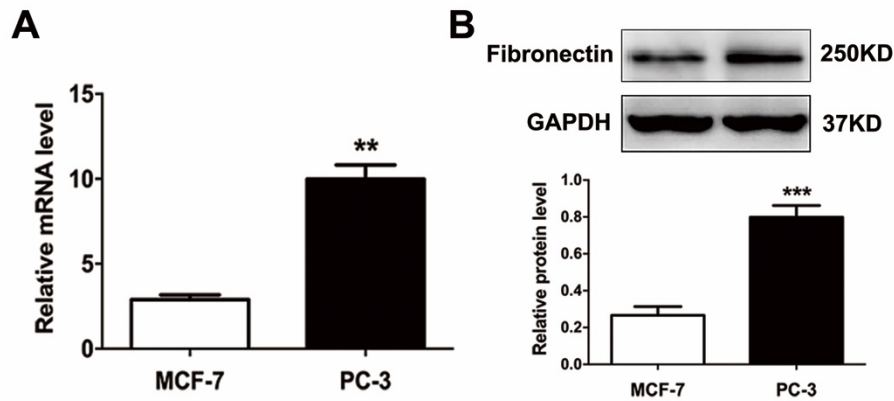
GAPDH PCR primers are listed as follow:

GAPDH F: 5'-GACACCCACTCCTCCACCTTT-3'

GAPDH R: 5'-CTCTCTTCCTCTTGTGCTCTTGC-3'

## Western blot

The proteins were separated by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore, USA). The membrane was blocked by 5% non-fat milk in TBST for 2 h and incubated with primary antibodies at 4 °C overnight. After washing thrice in TBST for 5 min each time, the membrane was incubated with labeled secondary antibody at room temperature for 1 h. The proteins were detected using the Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA) and images were acquired with the Bio-Rad System. Antibodies against fibronectin were provided by Abcam. GAPDH was used as an internal control.



**Fig. S7** Expression of fibronectin in PC-3 and MCF-7 cells. (A) mRNA level of fibronectin using qRT-PCR. (B) Representative western blots and quantification of fibronectin expression. \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , vs the MCF-7 cell.

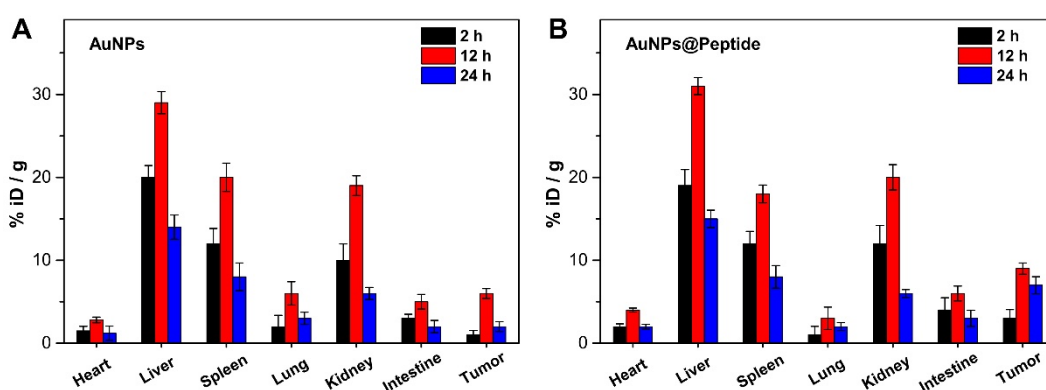
## 7. Animal experiments

### Animals and tumor implantation

The *in vivo* study was performed according to a protocol approved by the Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. A total of 20 male BALB/c nude mice (5 weeks old, 18-20 g) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China) and maintained in a specific pathogen-free environment. Each mouse was subcutaneously injected with a mixture of 0.2 mL PBS containing  $10^7$  cells into the right hind flank. Lengths and widths of tumours were measured individually using a vernier caliper. Tumour volumes were calculated using the following formula: tumour volume = length  $\times$  width<sup>2</sup>  $\times$  0.5.

## Inductively coupled plasma mass spectrometry (ICP-MS)

When the tumours reached a mean volume of 100 mm<sup>3</sup> after inoculation of PC-3 cells, the mice were randomly separated into two groups (n=3 per group): (1) AuNPs at 2 mg/mL; (2) AuNPs@Peptide at 2 mg/mL. The heart, liver, spleen, lung, kidneys, intestine and tumours were dissected at 2 h, 12 h, and 24 h after administration of AuNPs or AuNPs@Peptide through tail vein, respectively. All the samples were completely digested in acid (3:1 mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>) on a hot plate prior to ICP-MS analysis.



**Fig. S8** Biodistribution (heart, liver, spleen, lung, kidney, intestine and tumor) of AuNPs, AuNPs@Peptide in the PC-3 tumor-bearing mice at different post-injection time points (2 h, 12 h, and 24 h).

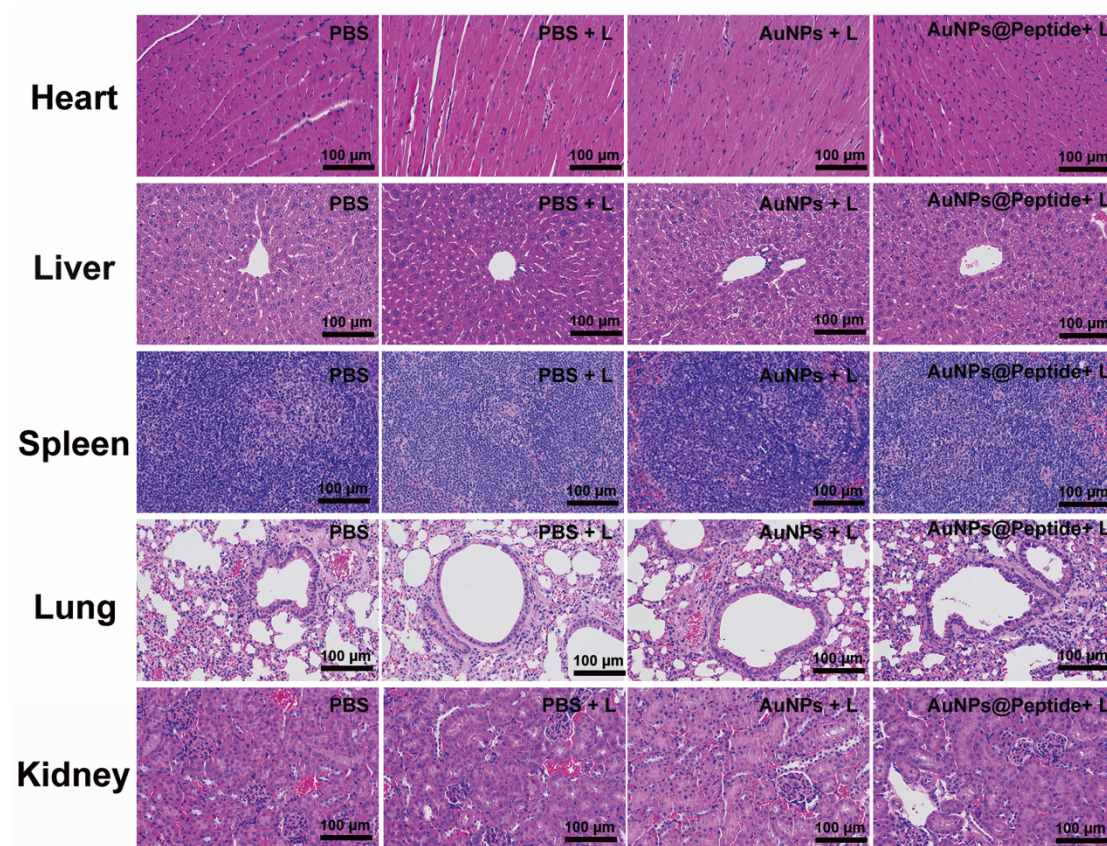
## *In vivo* antitumor studies

When the tumours reached a mean volume of 100 mm<sup>3</sup> after inoculation of PC-3 cells, the mice were randomly separated into four groups (n=5 per group): (1) PBS; (2) PBS with laser; (3) AuNPs at 10 mg/kg with laser; (4) AuNPs@Peptide at 10 mg/kg with laser. The power density of 650 nm laser for PTT was 5 W/cm<sup>2</sup> and the exposure time was 10 min. During therapy, the tumour volumes and body weights were measured every two days. The mice were sacrificed till 14 d post-treatment according to institutional guidelines. Tumours were resected, weighed, fixed in formalin and embedded in paraffin. The therapeutic efficacy of the treatment was evaluated by the tumour-inhibition rate (TIR). This was calculated using the following equation: TIR (%) = (mean tumour weight of PBS group - mean tumour weight of experimental group)/mean tumour weight of PBS group × 100.

## Histological examination of organs and tumours

Freshly dissected hearts, livers, spleens, lungs, kidneys and tumours from mice of four groups were fixed and embedded in paraffin. After being cut into 4 μm slices, the sections were

deparaffinized and stained with Hematoxylin and Eosin solution. The scan was used with a microscope (Nikon, Japan).



**Fig. S9** Histological changes of hearts, livers, spleens, lungs, kidneys in mice with PBS, PBS + L, AuNPs + L, AuNPs@Peptide + L treatment, respectively. L represent of laser at 650 nm (5 W/cm<sup>2</sup>). Scale bar: 100 μm.

## 8. Statistical analysis

The data was compared using GraphPad Prism version 5.0 (GraphPad Software, Inc).  $P < 0.05$  was considered statistically significant.