Supplementary Information

Title: Rational Design of a Prodrug to Inhibit Self-inflammation for Cancer Treatment

Xiaohui Zhu[a], Wen Han[a], Yongfei Liu[a], Haihui Wang[a], Danying Lin[a], Zhangcheng Fu[a], Yu He[a]*, Xiaofei Yin[b], Chunhua Lu[a]*, Huanghao Yang[a]

[a] MOE Key Laboratory for Analytical Science of Food Safety and Biology; Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety; State Key Laboratory of Photocatalysis on Energy and Environment College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, P. R. China

[b] First Institute of Ocanography, Ministry of Natural Resources, Qingdao 266061, P. R. China

* Corresponding author. E-mail: heyu@fzu.edu.cn ; chunhualu@fzu.edu.cn

Experimental Section

1. Preparation of Au-CZTS nanoparticles

In a typical synthesis, 460 mg of $CuCl_2 \cdot 2H_2O$, 195 mg of ZnO and 315 mg of $SnCl_4 \cdot 5H_2O$ were dissolved in 1 mL of the tetrahydrofuran (THF). Afterward, 8 mL of distilled OLA and 25 mL distilled 1-octadecene were added to the reaction mixture. The solution was heated under flow of argon to 175 °C and maintained at this temperature for 1 h to remove low boiling point impurities and water. The mixture was then cooled to 100 °C and 12 mL of tert-dodecylmercaptan and 1.2 mL of dodecanethiol were added by a syringe. Then the solution was heated to 250 °C and maintained at this temperature for 1 h. Finally, the CZTS nanocrystals were thoroughly purified by multiple precipitation and re-dispersion steps using 2-propanol and chloroform.

$AuCl_3$ (40 mg), DDAB (40 mg) and DDA (140 mg) were dissolved in toluene (4.0 mL) and sonicated for 30 min, until the colour of the gold precursor solution turned from dark orange to light yellow. This reaction mixture was added drop-wise to the CZTS nanocrystals dispersed in toluene (20 mL) at a rate of 80 mL/h for 5 min at room temperature. After that, the mixture was stirred for 4 h at 500 rpm. The Au-CZTS was immediately precipitated by adding methanol with centrifugation and finally dispersed in chloroform.

2. The photostability of Au-CZTS/Asp

First, the Au-CZTS/Asp was added into PBS for 20 days and 50 days. Meanwhile, the xenon lamp irradiated the Au-CZTS/Asp solution of 200 μg/mL at the power density of 10 $W \cdot cm^{-2}$ for different time. The TEM images of the solution after irradiation were recorded.

3. The photothermal transduction efficiency

From an energy balance on the system, the photothermal transduction efficiency could be calculated [1]. The total energy balance for the system is

$$\sum_i m_i c_{p,i} \frac{dT}{dt} = Q_{in,np} + Q_{in,surr} - Q_{out} \qquad (1)$$

where m and C_p are the mass and heat capacity of the solvent (water) and T is the solution temperature. $Q_{in,np}$ is the photothermal energy input from the nanocrystals

$$Q_{in,np} = I(1-10^{(-A_\lambda)})\eta \qquad (2)$$

where I is the laser power (in units of mW), A_λ is the absorbance at the excitation wavelength of 808 nm, and η is the photothermal transduction efficiency, or the fraction of absorbed light energy that is converted to heat. $Q_{in,surr}$ is the heat input (in units of mW) due to light absorption by the solvent, which was measured independently and found to be 25.1 mW. Q_{out} is the heat lost to the surroundings:

$$Q_{out} = hA(T - T_{surr}) \quad (3)$$

where h is the heat transfer coefficient, A is the surface area of the container, and T_{surr} is the ambient temperature. The lumped quantity hA was determined by measuring the rate of temperature drop after removing the light source. In the absence of any laser excitation, eq 1 becomes

$$\sum_{\mathrm{i}} m_i c_{p,i} \frac{dT}{dt} = -Q_{out} = -hA(T - T_{surr}) \quad (4)$$

Rearranging eq 4

$$dt = -\frac{m_{H_2O} C_{p,H_2O}}{hA} \frac{dT}{(T - T_{surr})} \quad (5)$$

and integrating, gives the expression

$$t = -(\frac{m_{H_2O} C_{p,H_2O}}{hA}) \ln(T - T_{surr}) \quad (6)$$

A characteristic rate constant can then be defined, $\tau_{out} = m_{H2O} C_{p,\,H2O}/hA$, such that

$$T - T_{surr} = \exp(\frac{hA}{m_{H_2O} C_{\mathrm{p},H_2O}} t) \exp(-\frac{t}{\tau_{out}}) \quad (7)$$

hA is the heat transfer coefficients were found for each solution during solution heating and cooling. At the maximum steady-state temperature, the rate of photothermal heating is then equal to the rate of heat transfer out of the system:

$$Q_{in,np} + Q_{in,surr} = I(1 - 10^{(-A\lambda)})\eta + Q_{in,surr} = hA(T - T_{surr}) \quad (8)$$

where T max is the maximum steady-state temperature. Therefore, the photothermal transduction efficiency can be calculated directly from the steady-state temperature increase, since

$$\eta = \frac{hA(T_{\max} - T_{surr}) - Q_{in,surr}}{I(1 - 10^{(-A\lambda)})} \quad (9)$$

4. The release curves of aspirin from Au-CZTS/Asp under different conditions

First, the standard curve of Asp was measured and obtained. The Au-CZTS/Asp solution was treated with 808 nm laser (1W·cm^{-2}) for 10 min and then centrifuged to determine the Asp released in the supernatant. In addition, 200μg/mL of Au-CZTS/Asp solution was incubated with PBS, FBS and cell lysate for different time and then centrifuged to determine the Asp released in the supernatant.

5. cGAMP mediated cell endocytosis

The lipofectamine™ 3000 (7.5 μL) was mixed with 250μL MEM ™ culture medium. Then, the cGAMP (5 μg), P3000™ (10 μL) and MEM ™ culture medium (250 μL) was mixed to form cGAMP solution. The diluted lipofectamine™ 3000 and cGAMP solution was further mixed for 15 min in proportion of 1:1. Subsequently, the cGAMP-lipofectamine™ 3000 incubated with cells.

6. *In vivo* experiments

Female BALB/c mice (6 weeks) were purchased from Shanghai SLA Laboratory Animal Co., Ltd. and used under protocols approved by the Institutional Animal Care and Use Committee of Fuzhou University. The 4T1 cells (1×10^6) suspended in D-PBS were subcutaneously injected into right hip of each female BALB/c mouse. The mice were treated when the tumor volume reached about 100 mm^3. The tumor-bearing mice were divided into 6 groups, 5 mice per group, to quantify the growth rate of tumors after the following treatments: (1) only PBS; (2) PBS + 1 W·cm^{-2} 808 nm NIR laser irradiation for 10 min; (3) only Au-CZTS (5mg/kg); (4) Au-CZTS (5mg/kg) + 1 W·cm^{-2} 808 nm NIR laser irradiation for 10 min; (5) only Au-CZTS/Asp (5mg/kg); (6) Au-CZTS/Asp (5mg/kg) + 1 W·cm^{-2} 808 nm NIR laser irradiation for 10 min. The whole operation was performed every 2 days for 14 days. Meanwhile, the weight of mice and the volume of the tumors were measured by calliper and weight scale every day for 14 days. The tumors and major organs (heart, liver, spleen, lung, kidney) were dissected after PTT and examined by Hematoxylin-eosin (H&E) and DAPI-TUNEL staining.

7. The distribution and clearance efficiency of Au-CZTS/Asp *in vivo*

For *in vivo* distribution studies, the mice bearing 4T1 tumor were sacrificed after intravenous injection of Au-CZTS/Asp (5mg/kg) 0 h, 6 h, 12 h,18 h and 24 h, respectively. The main organs, such as heart, liver, spleen, kidney, lung and tumor were collected and further digested to measure the content of Zn by ICP-MS.

Furthermore, the clearance efficiency of Au-CZTS/Asp was evaluated in the mice. After the mice was injected Au-CZTS/Asp, feces and urine sample were collected at 0 h, 1 h, 2 h, 4 h, 6 h, 12 h, 18 h, and 24 h, respectively. After weighing the wet samples, these samples were decomposed and further the content of Zn was analysed by ICP-MS. In addition, the urine sample were collected and further measured the TEM to evaluate the stability of Au-CZTS/Asp.

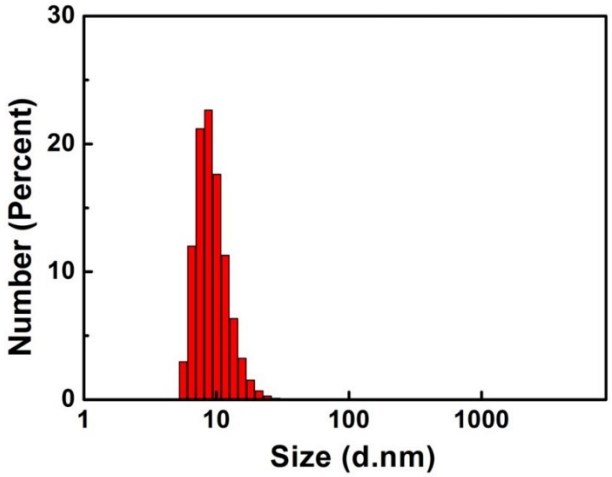

Figure S1. The DLS of Au-CZTS.

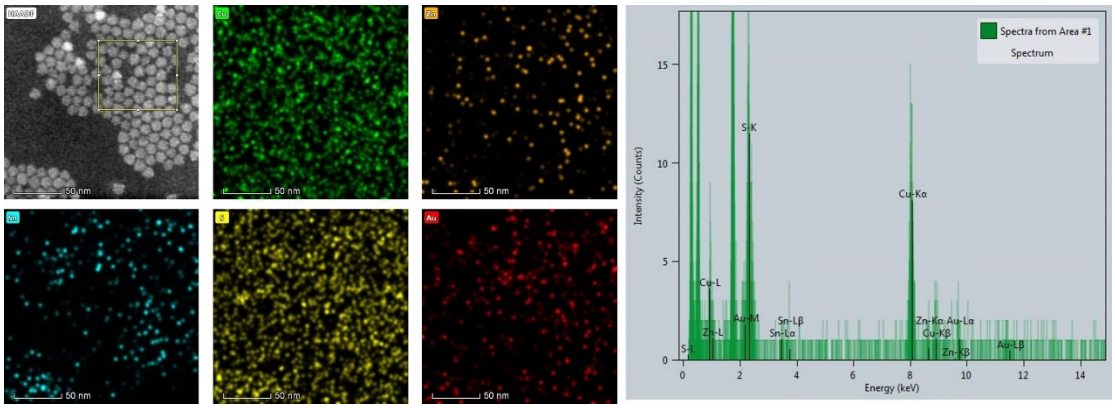

Figure S2. The element distribution of Cu, Zn, Sn, S and Au of Au-CZTS.

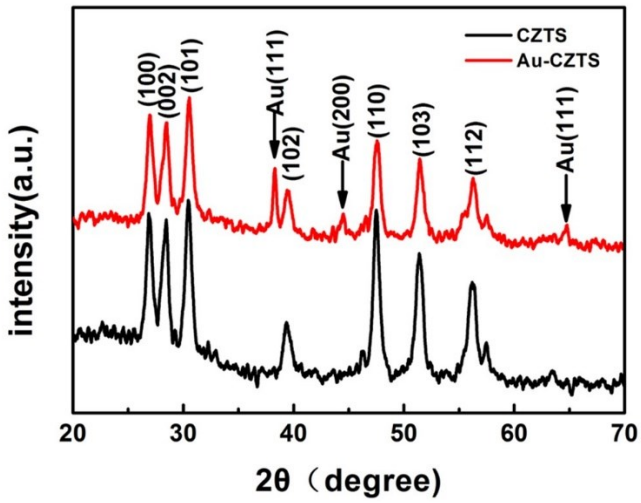

Figure S3. The XRD pattern of CZTS and Au-CZTS nanocrystals.

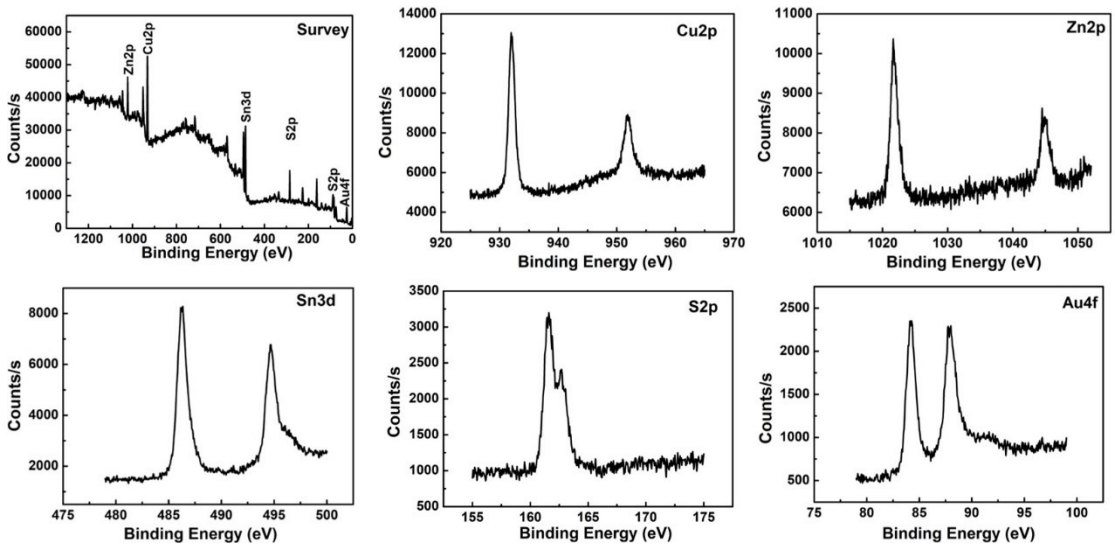

Figure S4. The XPS pattern of Au-CZTS nanocrystals and the characteristic peak of Cu2p, Zn2p, Sn3d, S2p and Au4f.

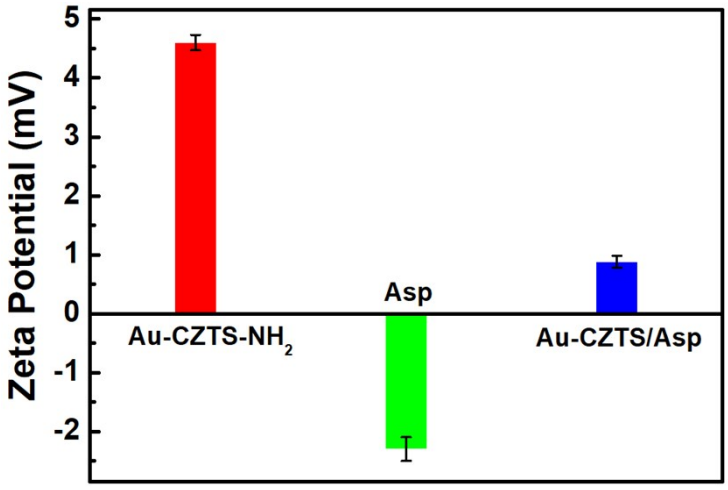

Figure S5. The zeta potential of Asp, Au-CZTS-NH_2 and Au-CZTS/Asp. (n=3)

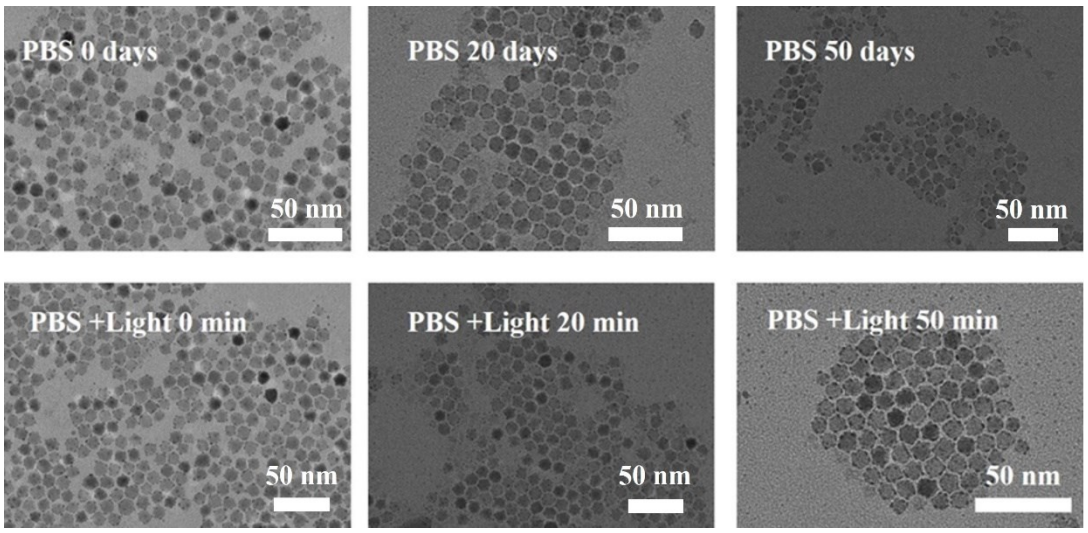

Figure S6. The TEM image of Au-CZTS was treated with different means to evaluate the stability of the Au-CZTS in the PBS.

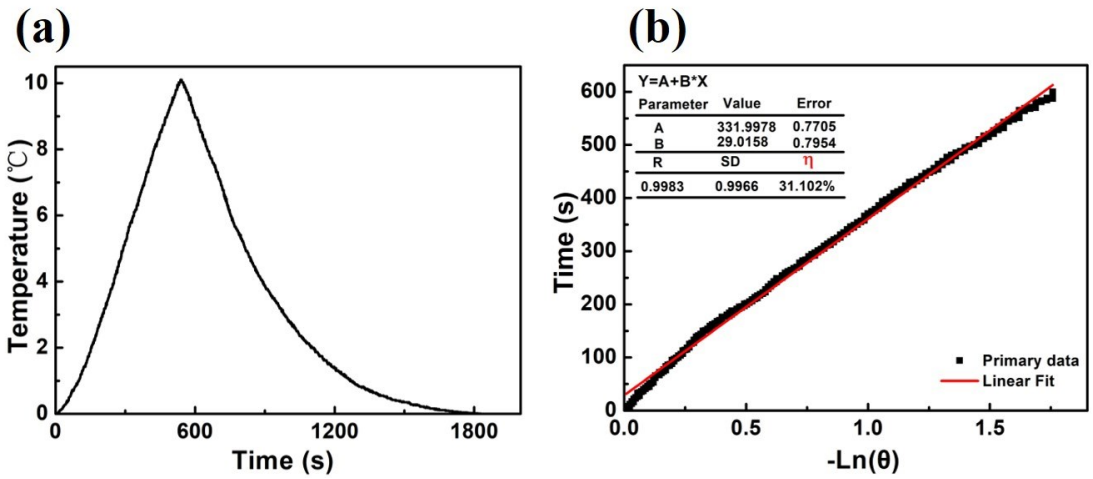

Figure S7. (a) and (b) Heating and cooling curves and photothermal conversion efficiency of CZTS.

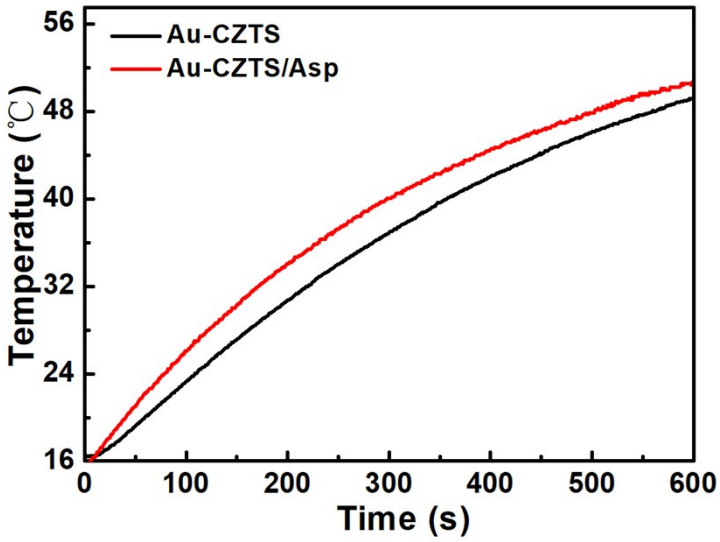

Figure S8. The photothermal properties of Au-CZTS and Au-CZTS/Asp.

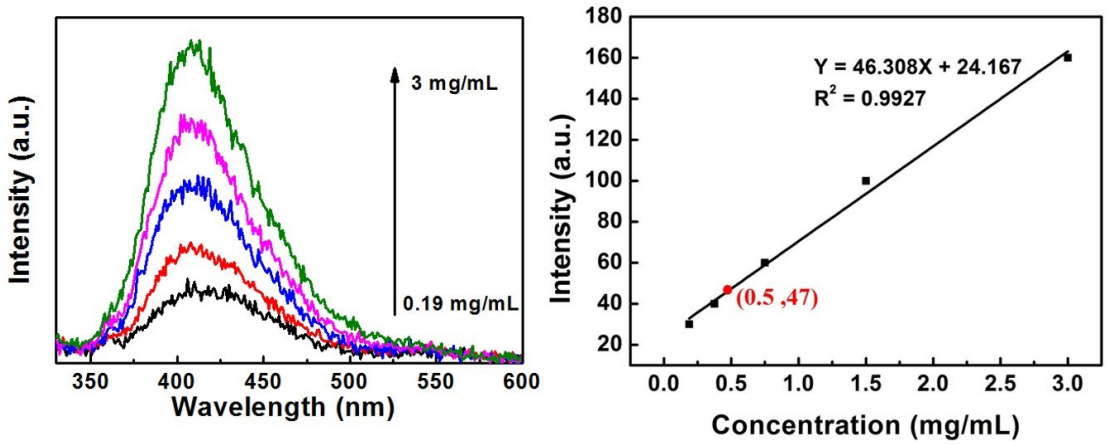

Figure S9. The fluorescence of Asp at different concentration and the standard curve of Asp. Briefly, 8 mg of Asp and 50 mg of Au-CZTS-PEG were mixed for 12 h to prepare the Au-CZTS/Asp. After the mixture was centrifuged to remove the Au-CZTS/Asp precipitate, the supernatant was obtained. The absorb of supernatant at 410 nm wavelength was 47, which was record by ELIASA. Based on the absorb of supernatant and the standard curve of Asp, we can obtain the concentration of Asp in supernatant was 0.5 mg/mL. Therefore, the Asp loading on the Au-CZTS was 5.5 mg and the loading capacity of Asp was computed from the following equation: 5.5/ (5.5+50) = 9.91 %.

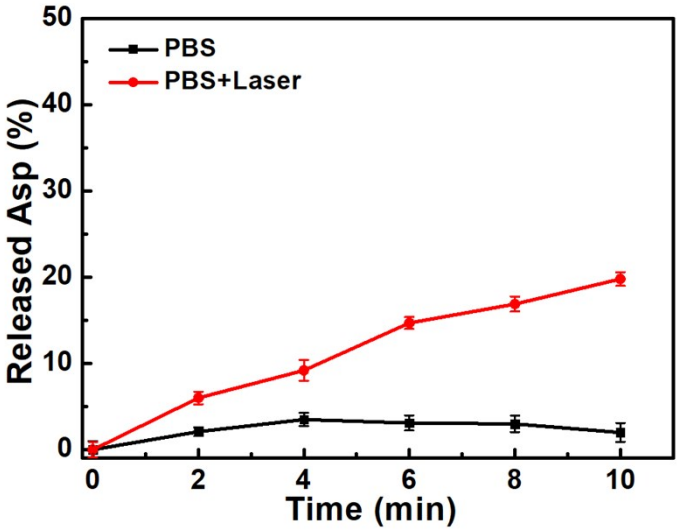

Figure S10. The Asp release of Au-CZTS/Asp in PBS with/without light.

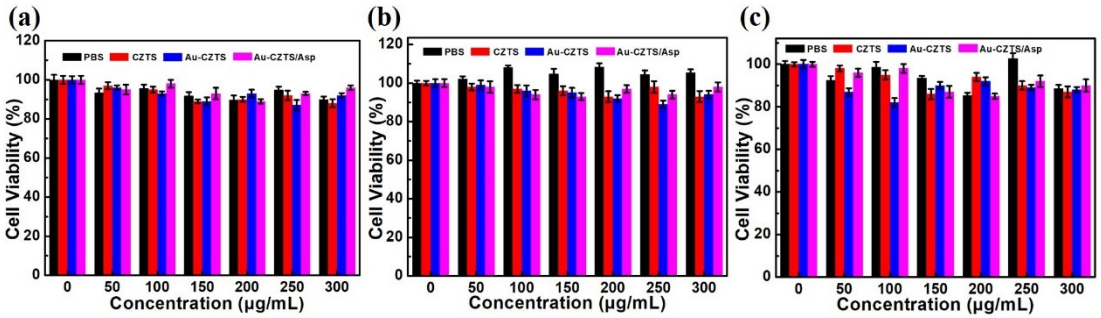

Figure S11. The biocompatibility and cytotoxicity of different samples were incubated with (a) 4T1, (b) L02 and (c) MCF-7. (n=5)

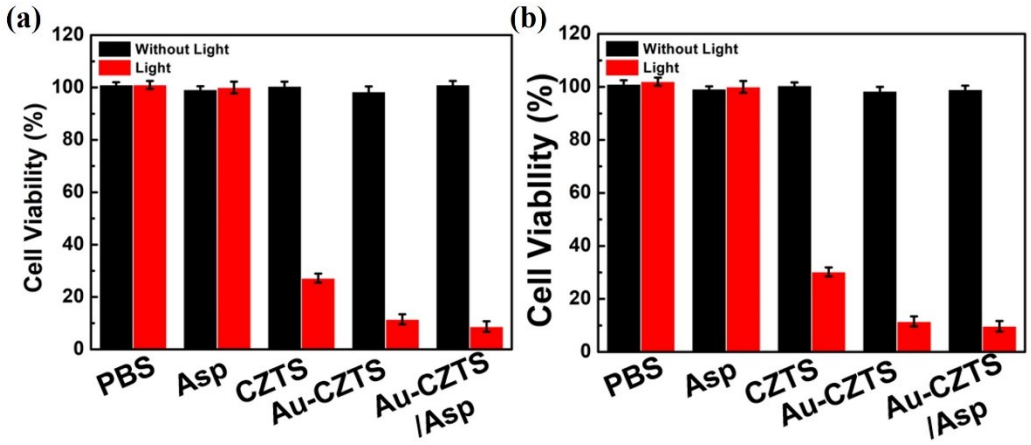

Figure S12. The cell viability of (a) MCF-7 and (b) L02 cells with different treatments. (n=5)

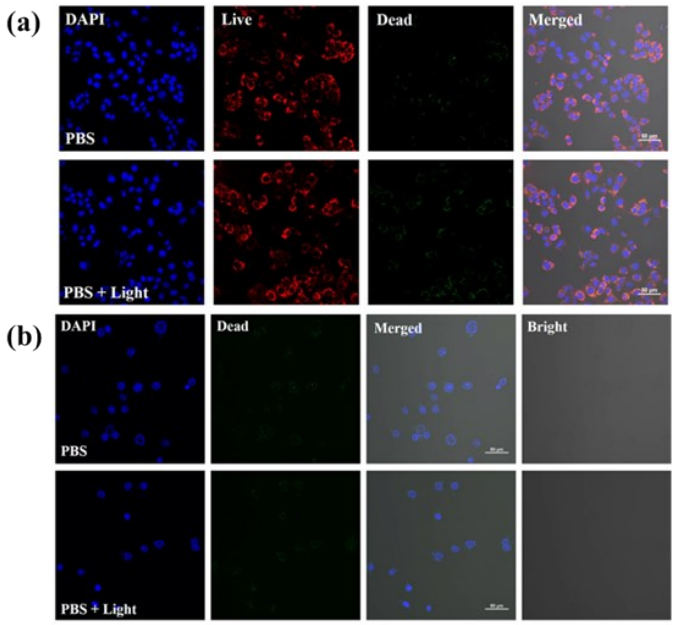

Figure S13. The control group of (a) the mitochondrial membrane potential and (b) TUNEL assay.

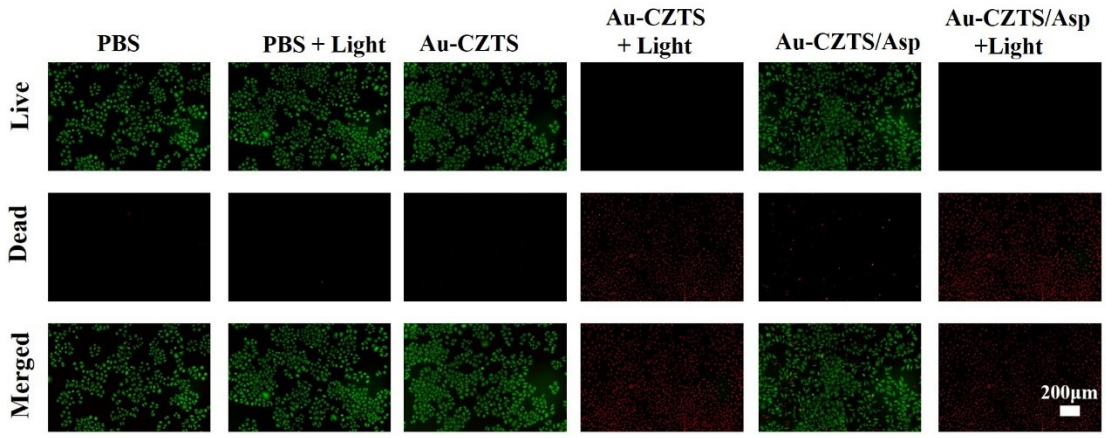

Figure S14. The fluorescence images of Calcein AM and PI-costained cells with different treatments using 4T1 cells incubated with different samples at a concentration of 150 μg/mL under irradiation (808 nm, 1 W·cm^{-2}, 10 min).

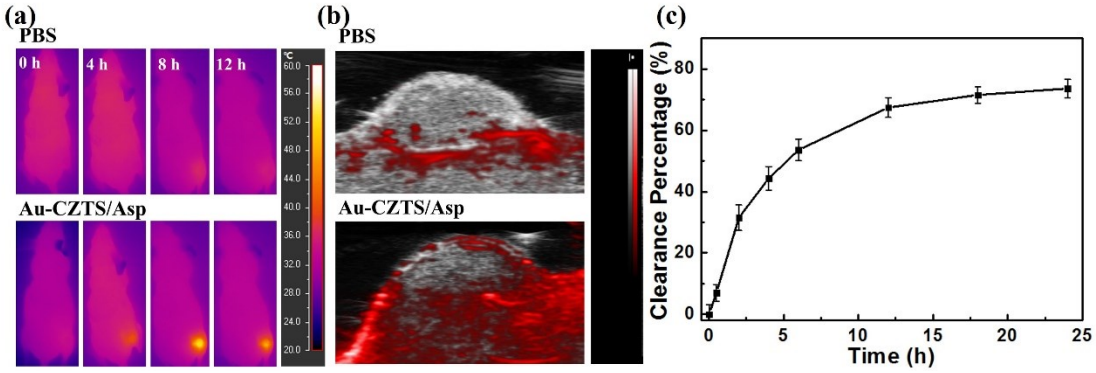

Figure S15. (a) IR Thermal imaging and (b) PA signal of tumor-bearing mice after injected with PBS and Au-CZTS/Asp, respectively. (c) The clearance efficiency of Au-CZTS/Asp *in vivo*.

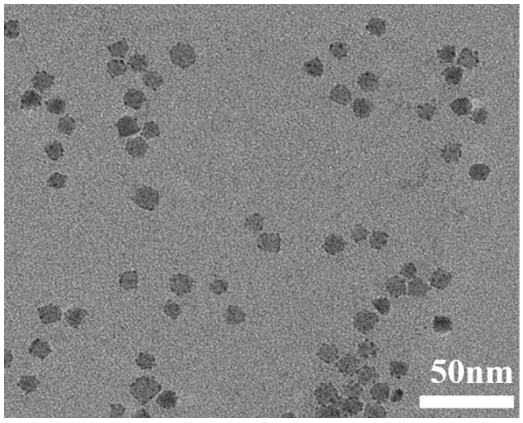

Figure S16. The TEM image of Au-CZTS was collected form the mouse urine.

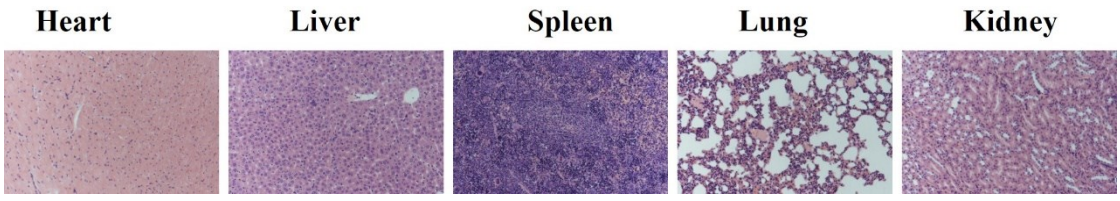

Figure S17. The tissue slice of heart, liver, spleen, lung and kidney.

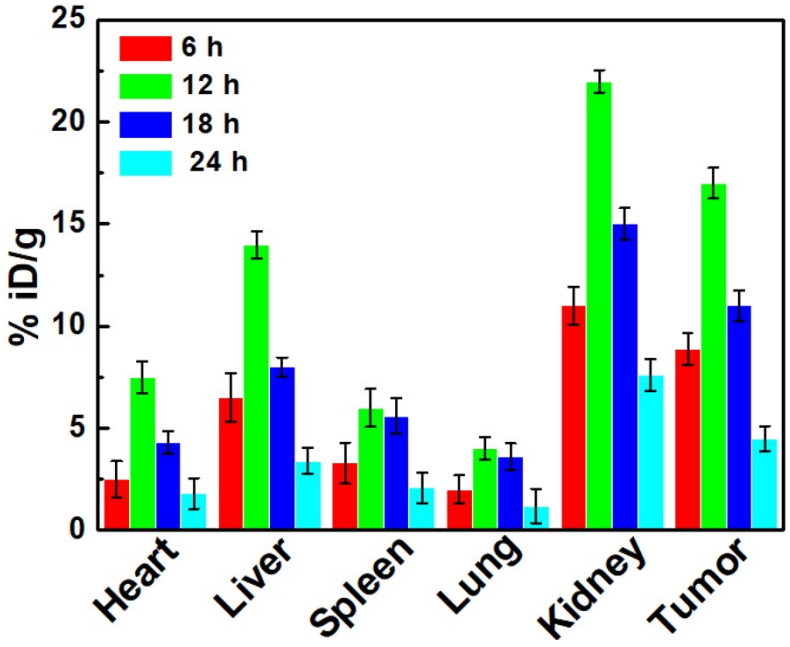

Figure S18. The tissue slice of heart, liver, spleen, lung and kidney.

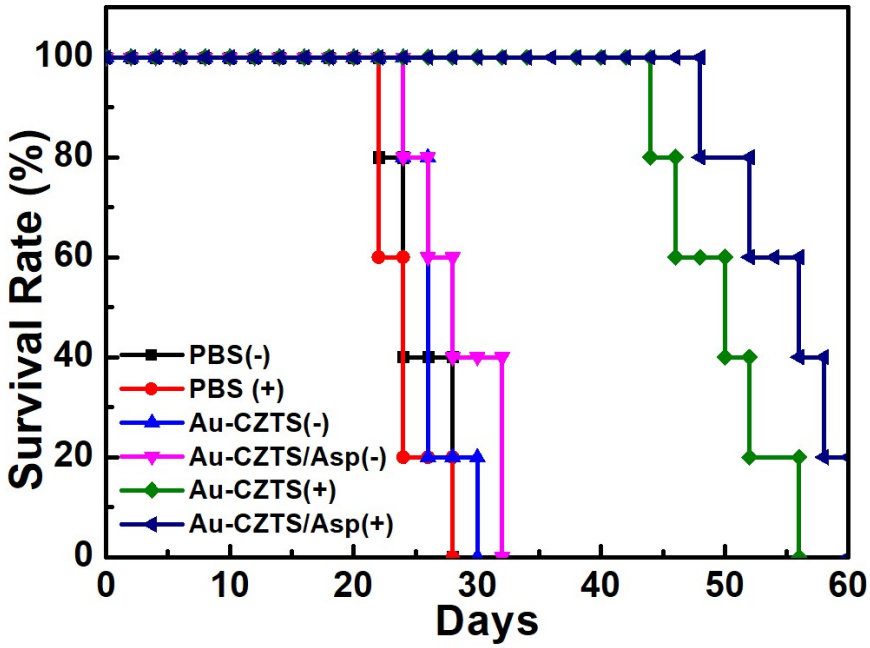

Figure S19. The survival rate for different groups. (n=5)

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