Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Theranostic Probe for Simultaneous *in vivo* Photoacoustic Imaging and Confined Photothermolysis by Pulsed Laser at 1064 nm in 4T1 Breast Cancer Model

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Methods

a. Stability of CuS NPs in different media.

The stability of PEG-CuS NPs in different media were investigated by incubating PEG-CuS NPs in phosphate-buffered saline (PBS) and PBS containing 10% fetal bovine serum (FBS) at 37 °C for 7 days. UV-Vis spectra were acquired at day 7.

b. Preparation of PEG-[^{64}Cu]CuS NPs.

$^{64}CuCl_2$ (20 μL, 1000 μCi) was added to 1 mL of $CuCl_2$ solution (1 mM) containing PEG-SH (2 μmol), after which 1 μL of sodium sulfide solution (1 M) was added to the [^{64}Cu]$CuCl_2$ solution with stirring. The mixture was then heated to 90 °C for 15 min until a dark-green solution was obtained. The reaction mixture was transferred to ice-cold water to give PEG-[^{64}Cu]CuS NPs.

c. Photoacoustic (PA) imaging equipment

A Q-switched Nd:YAG laser (LS-2137, Symphotic Tii, Camarillo, CA, USA) provides 10-ns, 355-mJ pulsed 1064-nm laser at a repetition rate of 10 Hz. The laser beam is expanded by a concave lens and homogenized by a ground-glass lens and then directed onto a mouse tumor with beam size of 2 cm in diameter. NIR light diffuses inside a tissue sample and induces PA waves. The waves travel through the tissue and are coupled to an unfocused ultrasonic transducer with a 6-mm-diameter active element and 1.8-MHz detection bandwidth (V323/2.25 MHz, Panametrics, Waltham, MA, USA), which converts the PA pressure into piezoelectric signals. The signals are subsequently amplified by amplifiers (ZFL-500LN, Mini-Circuits, and 5072PR, Panametrics, Waltham, MA, USA), bandpass-filtered by our homemade filters, and finally recorded using a digital data acquisition card (CS14100, Gage Applied, Inc., Lockport, IL, USA). The sample and a transducer are both immersed in a tank filled with water for coupling the PA waves to the transducer. The ultrasonic transducer is driven by a step motor to

continuously scan horizontally along a 15-cm-diameter circle around the sample. A personal computer is used to control the scanning and data acquisition.

d. Copper staining in 4T1 tumors

A stock solution of 0.2% 5-(p-dimethylaminobenzyli-dine) rhodanine (JT Baker Company, Phillipsburg, NJ) was prepared by mixing 0.2 g of the compound in 100 mL absolute ethanol. A working solution of rhodanine was then prepared by mixing 20 mL of the stock solution with 30 mL of deionized water. Tissue sections that had been de-paraffinized and rehydrated to deionized water were placed in the rhodanine working solution in a 60°C oven for 1 h. They were then rinsed four times in deionized water, counter-stained with Mayer's hematoxylin for 10 minutes, and rinsed another three times in deionized water. After brief bluing in 0.5% sodium borate solution, they were rinsed another three times in deionized water, dehydrated and cleared with two changes each of 95% ethanol, absolute ethanol, and xylene, and mounted with a resinous medium.

e. Study of temperature changes in aqueous solution of CuS NPs under 1064-nm and 808-nm NIR laser light

For measurement of temperature change mediated by CuS NPs, continuous NIR laser light (808 nm, 3 W/cm^2, Diomed, Andover, MA) or pulse laser light (1064 nm, 4.32 W/cm^2) was delivered through a quartz cuvette containing CuS NPs (100 μg/mL). A thermocouple was inserted into the solution perpendicular to the path of the laser light.

f. Statistical Analysis

Differences in necrosis (expressed as percentage of necrotic area after treatments) were analyzed using the two-tailed Student's *t* test. Differences between groups were considered statistically significant at $p < 0.05$.

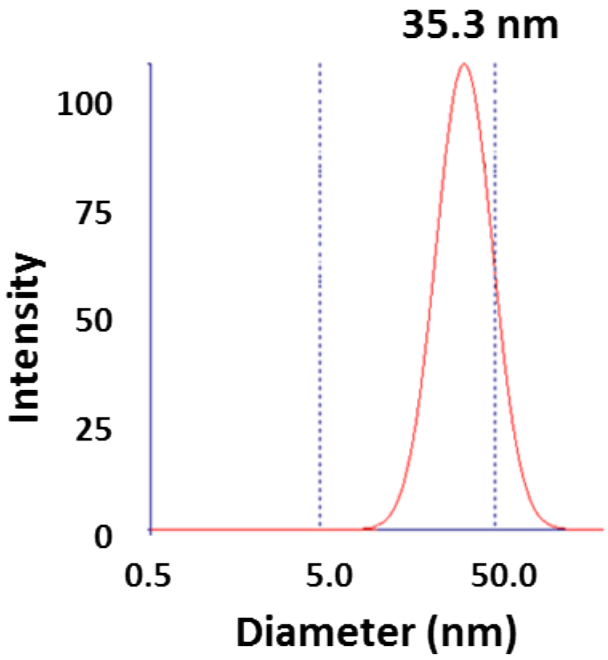

Figure S1. Dynamic light scattering of PEG-CuS NPs at 6 months after storage in water at 4 ºC in the presence of argon. The size of the nanoparticles was essentially unchanged compared to that of freshly prepared nanoparticles (Fig. 1C).

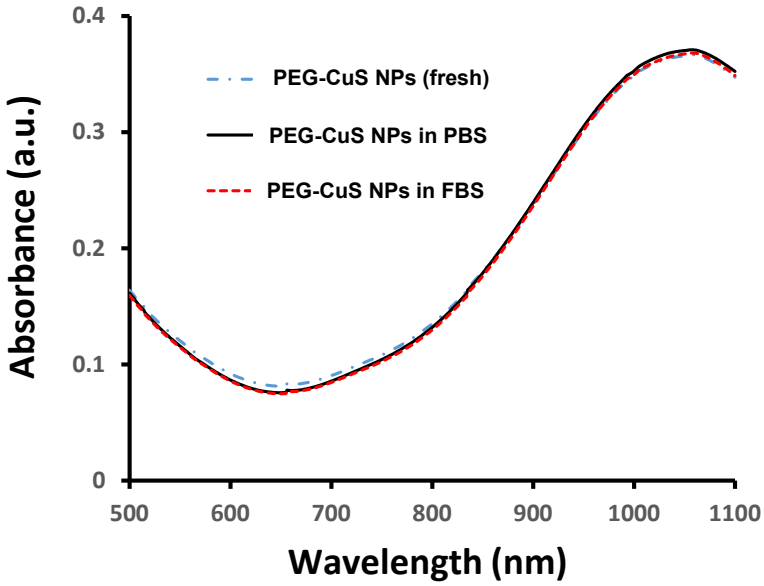

Figure S2. UV-Vis spectra of PEG-CuS NPs after incubation in PBS and FBS media at 37 ºC for 7 days.

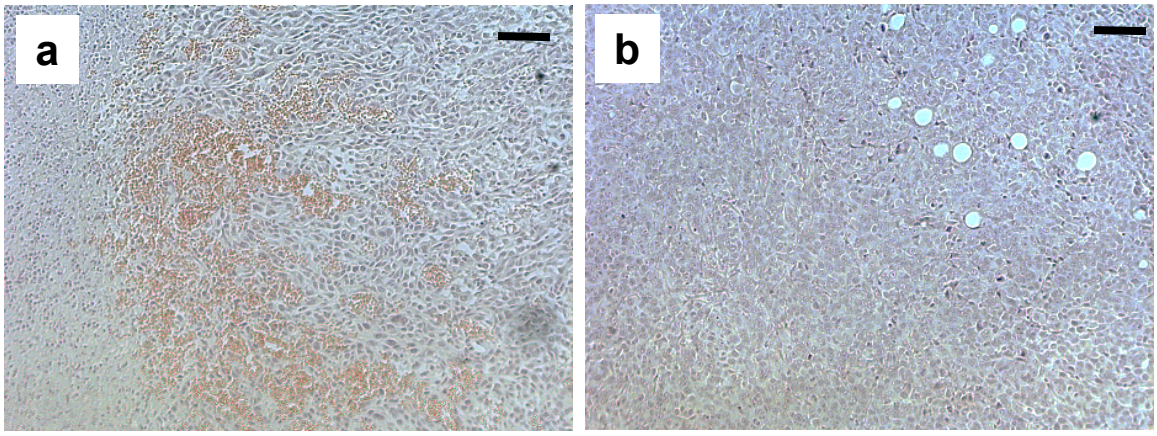

Figure S3. Copper staining of excised 4T1 tumors. (a) Copper was seen in a tumor from a mouse injected with PEG-CuS NPs. (b). No copper ions were detected in a tumor from a mouse that did not receive PEG-CuS NPs. Bar = 50 μm

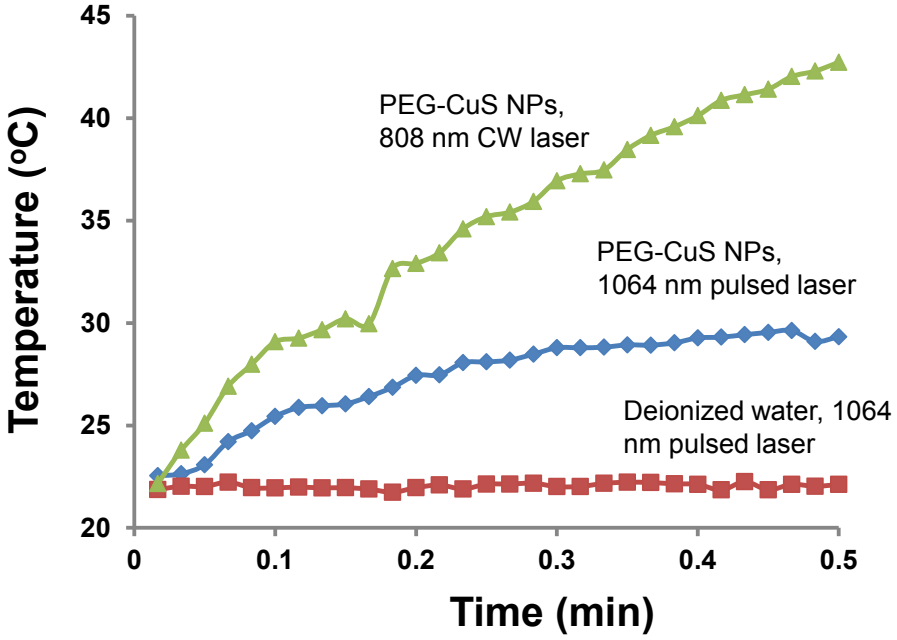

Figure S4. Temperature change after exposure of aqueous solution of PEG-CuS NPs (100 µg/mL) to continuous wave (CW) laser light at 808 nm (3 W/cm^2) and pulsed laser light at 1064 nm (4.32 W/cm^2). Deionized water was also exposed to pulsed laser at 1064 nm (4.32 W/cm^2) as a control. Temperature elevation was observed only for solutions containing PEG-CuS NPs. Exposure to CW laser generated more heat than exposure to pulsed laser.

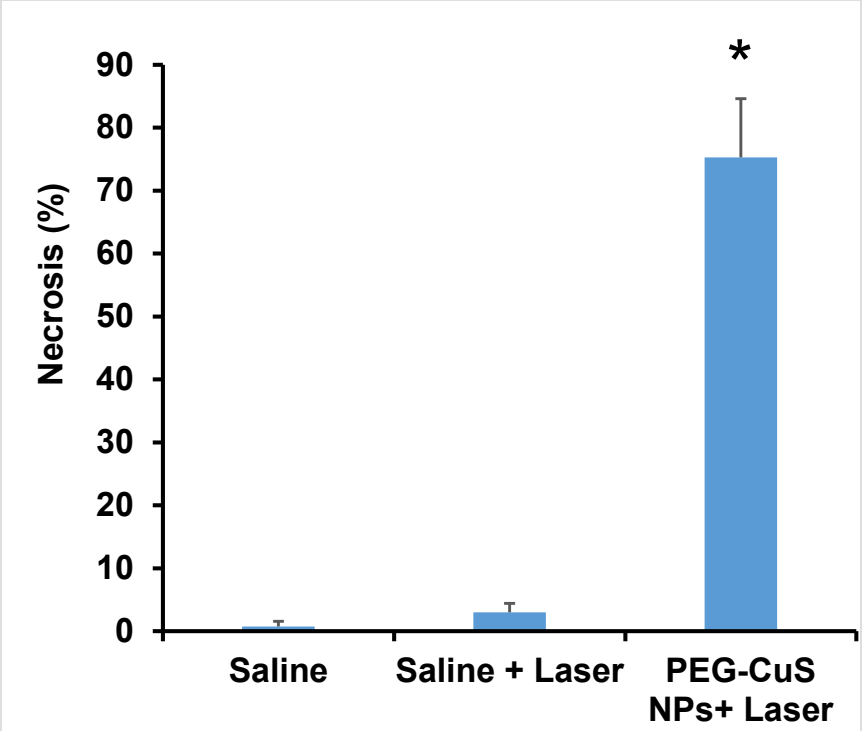

Figure S5. Quantitative analysis of the percentage of necrotic area induced by various treatments. Asterisks indicate statistical significance relative to the saline group ($p < 0.0001$) and saline group ($p < 0.0001$). Error bars represent standard deviations ($n = 4$).