Near-Infrared Emission CuInS/ZnS Quantum Dots: All-in-One Theranostic Nanomedicines With Intrinsic Fluorescence/Photoacoustic Imaging for Tumor Phototherapy

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Experimental Section

1. Photothermal performance in vitro

To measure the photothermal effect, 2mL of ZCIS NMs-25 aqueous solution in a quartz cuvette was irradiated by a 660 nm NIR laser at a power density of 1 W/cm^2 for 10 min. The real-time thermal imaging of the quartz was recorded every 30 s by a Fluke thermal camera (Ti-400).

Photothermal conversion efficiency (η) was calculated by Roper's method. The temperature change of the ZCIS NMs dispersion (200 μg/mL) was recorded under the continuous 660 nm laser irradiation (1.0 W/cm^2) until reaching a steady-state temperature. The η was calculated via Eq. (1):

$$\eta = \frac{\mathrm{hs}(T_{max}-T_{sur})-Q_{Dis}}{I(1-10^{-A_{660}})} \qquad (1)$$

where h is the heat transfer coefficient, s is the surface area of the container, T_{max} is the equilibrium temperature (48.1 °C), T_{sur} is the ambient temperature of the environment (26 °C), Q_{Dis} means the heat dissipation from the light absorbed by the quartz sample cell (0.23 mW), I is incident laser power (1 W/cm^2), and A_{660} is the absorbance of ZCIS NMs at 660 nm (0.5). The value of hs is derived according to Eq. (2):

$$\tau_s = \frac{m_D c_D}{hs} \qquad (2)$$

Where τ_s is the sample system time constant (322.1 s); m_D and c_D are the mass (1.0 g) and heat capacity (4.2 J/g) of the deionized water used as solvent, respectively. Therefore, hs was calculated to be 0.013.

2. Detection of ROS in ZCIS NMs solution

The detection of singlet oxygen ($^1O_2$) was performed following the Kraljic procedure. 2 mL solutions of p-nitrosodimethylaniline (RNO, 30 μM), imidazole (0.5 mM) and ZCIS NMs-25 (100 μg/mL) in PBS (10 mM, pH=7.4) was irradiated with 660 nm laser at the power density of 1 W/cm^2

for 25 min. The absorbance reduction of RNO at 440 nm was recorded by a UV-Vis absorption spectrophotometer every 5 min.

3. Cell line, multicellular spheroids, and animal.

Mice breast cancer 4T1 cells were routinely maintained in RPMI 1640 (Roswell Park Memorial Institute 1640, Gibco BRL) medium containing 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). The cells were cultured in tissue culture flasks in a humidified atmosphere containing 5% CO_2 at 37 ^{o}C.

Multicellular spheroids (MCSs) were cultured according to a reported method. Briefly, a T75 flask (Corning, USA) was covered by 10 mL of hot agarose solution (1.5 w/v %) and then cooled to room temperature. 4T1 cells were seeded at a density of 1 million cells per flask in 15 mL of RPMI 1640 medium and incubated for 4 days to grow into spheroids.

BALB/c nude mice (~20 g, 6 weeks, female) were purchased from Beijing HFK Bioscience Co., Ltd. The 4T1 xenograft tumor model was established by injecting 5 million 4T1 cells in 100 μL of PBS into the right flank of the nude mice. When the tumor volume reached ~100 mm^3, the treatment experiments were conducted in accordance with guidelines approved by the ethics committee of Peking University.

4. Cell viability assessment.

4T1 cells in the growth of log phase were cultured in 96-well plates at density of ~6000 cells/well and incubated for 24 h. 200 μL of ZCIS NMs-25 solutions diluted with fresh culture media was added to each well. After 24 h exposure to ZCIS NMs-25 solutions at various concentrations, the culture media were changed back to fresh culture media to remove free ZCIS NMs in the solution, and cell viability was investigated by standard MTT assays.

5. Photo-induced ablation in vitro and cellular ROS detection.

The phototherapy efficiency of ZCIS NMs was evaluated on 4T1 cells. 4T1 cells were first cultured into 96-well plates at a density of 6000 cells/well for 24 h, and washed with PBS solution for 3 times. 200 μL of ZCIS NMs-25 solutions (100 μg/mL) diluted with fresh culture media was added and incubated with cells for 8 hours, followed by washing step with PBS. The treated cells were exposed to a 660 nm laser at a power density of 1W/cm^2 for 10 min. Cell viability after laser irradiation treatment was evaluated by standard MTT assays. Upon treatment, the cells were co-stained with Calcein-AM and PI for live/dead cell staining, and then observed by a Zeiss LSM 710 inverted laser confocal scanning microscope.

The cellular ROS was detected by ROS probe DCFH-DA, which can de-esterified intracellularly and turns to fluorescent DCFH upon oxidation by ROS. 4T1 cells were cultured into 24-well plate and incubated for 24 h. After washed by PBS for 3 times, the cells were further incubated in fresh medium containing ZCIS NMs (100 μg/mL). After 8 hours incubation, the 4T1 cells were incubated with 10 μM DCFH-DA in serum free 1640 medium for 20 min. After washed 3 times with PBS, the cells were irradiated for 10 min and immediately observed by a Zeiss LSM 710 inverted laser confocal scanning microscope.

6. Statistical Analysis

Results were expressed as mean and SD. Two-tailed paired and unpaired Student's t tests were used to determine differences within groups and between groups, respectively. P value <0.05 was considered statistically significant.

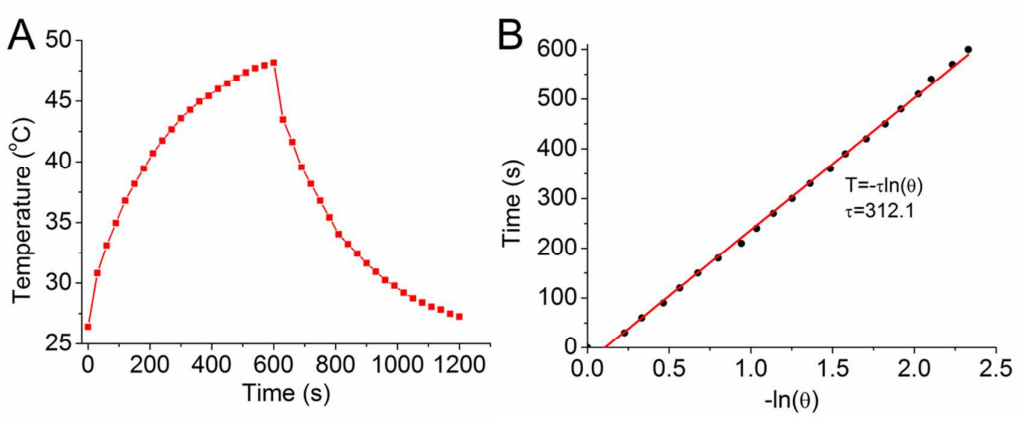

Figure S1. A, Photothermal effect of the ZCIS NMs aqueous solution (200 μg/mL) when illuminated with a 660 nm laser (1.0 W/cm^2). The laser was turned off after irradiation for 10 min. B, Plot of cooling time versus negative natural logarithm of the temperature driving force obtained from the cooling stage as shown in (A). The time constant for heat transfer of the system is determined to be τs = 312 s.

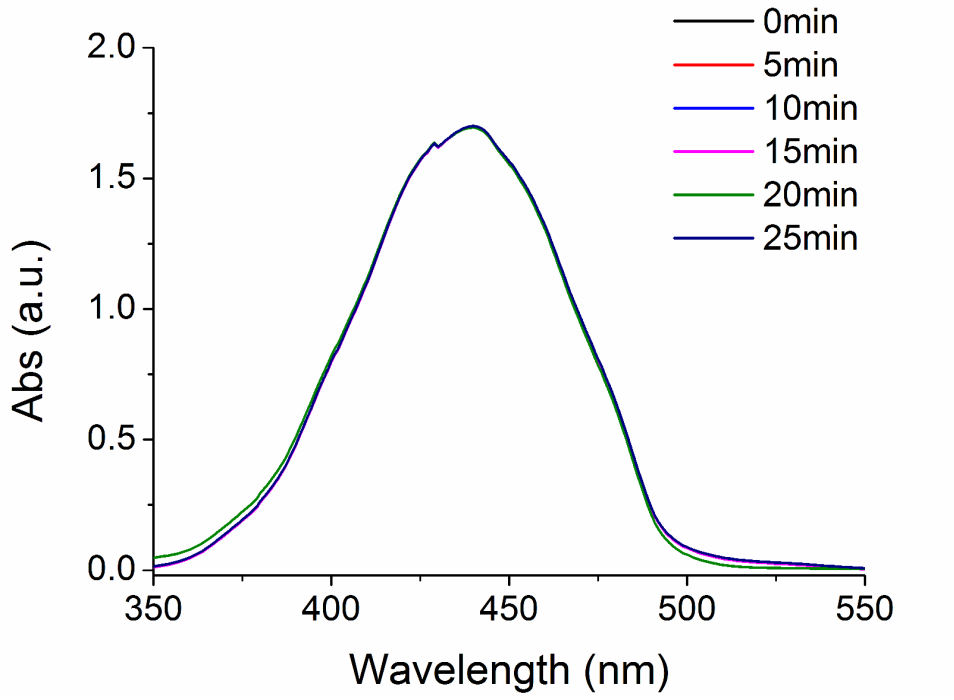

Figure S2. The absorbance spectrum of RNO solution without ZCIS NMs under laser the 660 nm laser irradiation.

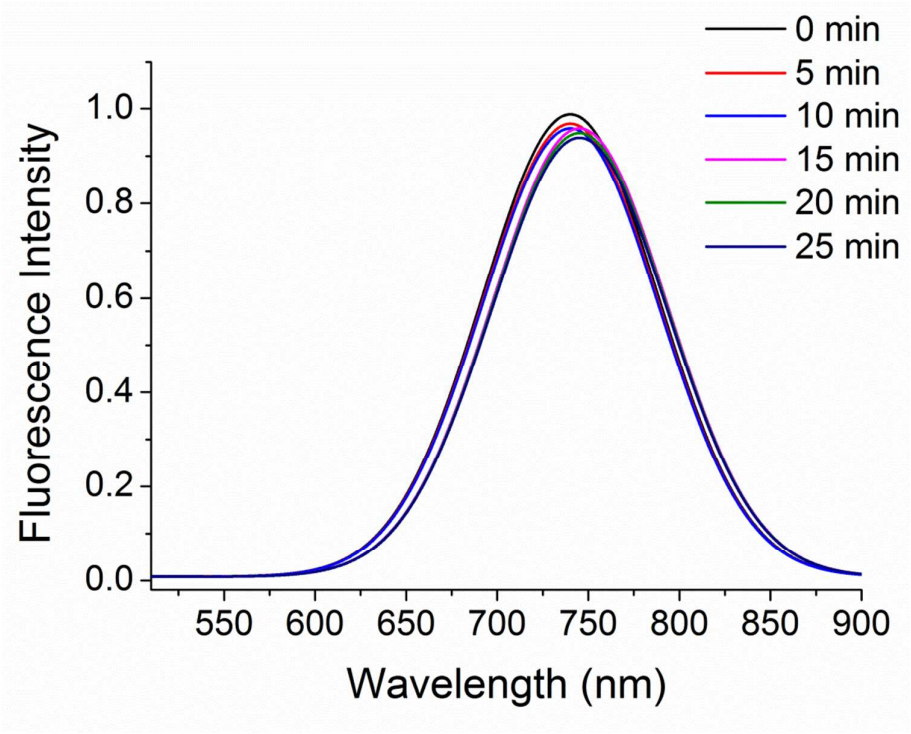

Figure S3. Fluorescence spectra of ZCIS NMs suspension under the 660 nm laser irradiation.

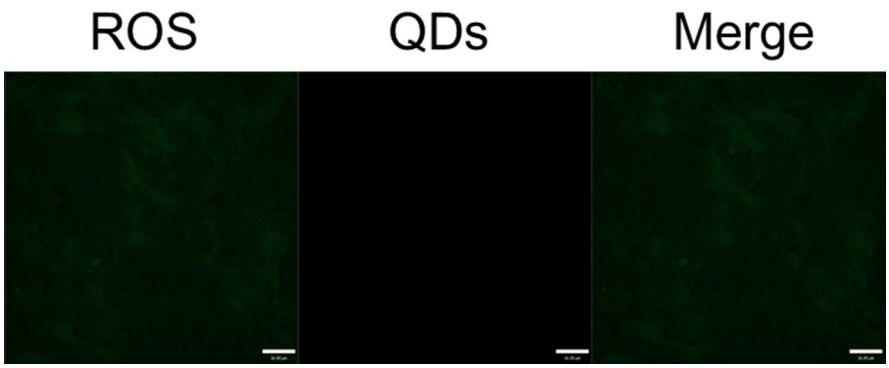

Figure S4. Confocal fluorescence images of cells treated with PBS only. Cellular ROS generation was detected by DCF fluorescence. Scale bar = 16 μm.

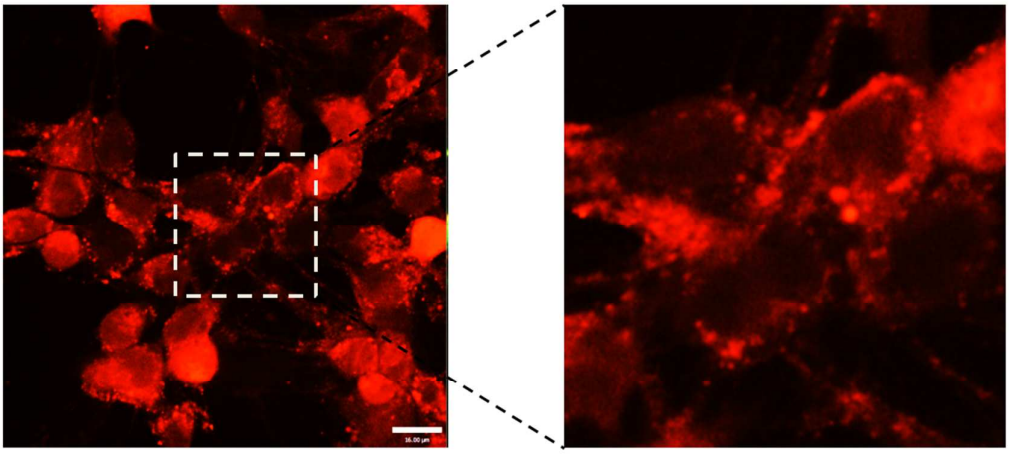

Figure S5. An enlarged confocal fluorescence image of cells treated with ZCIS NMs/laser (QDs+Laser). Red fluorescence displayed the inherent fluorescence of ZCIS NMs visualizing their intracellular distribution. Scale bar = 16 μm.

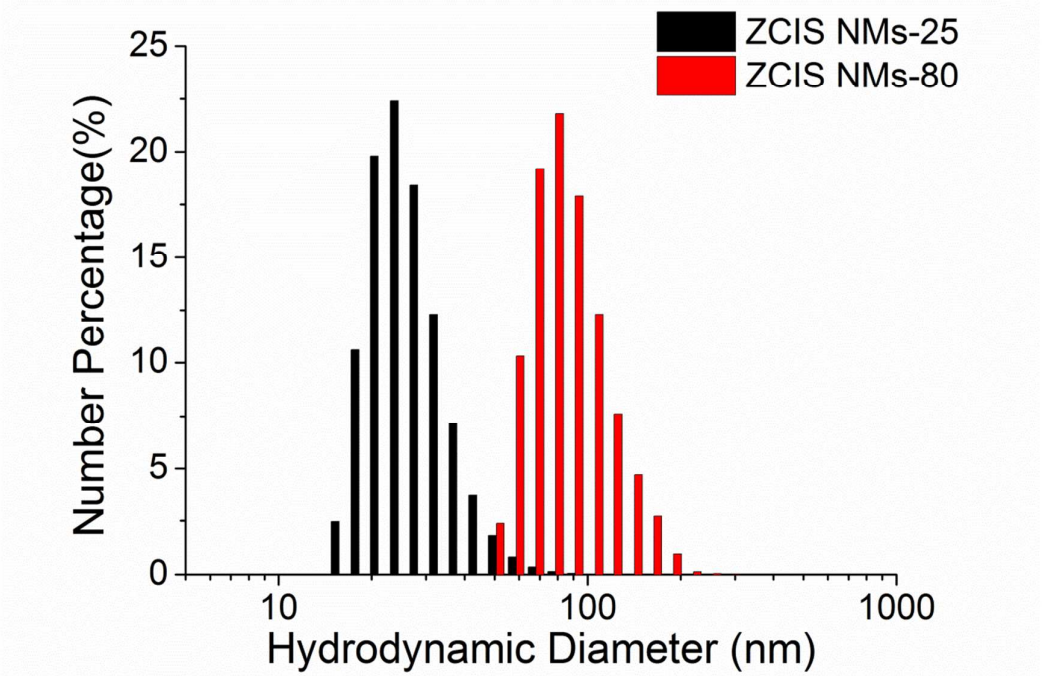

Figure S6. Hydrodynamic diameters distribution of ZCIS NMs-25 and ZCIS NMs-80 in PBS (1X), respectively.

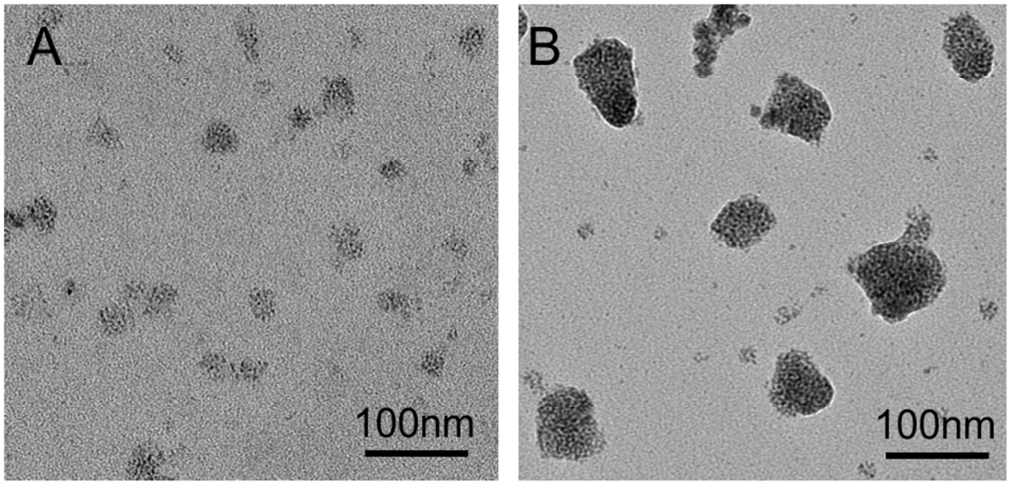

Figure S7. TEM images of ZCIS NMs-25 (A) and ZCIS NMs-80 (B).

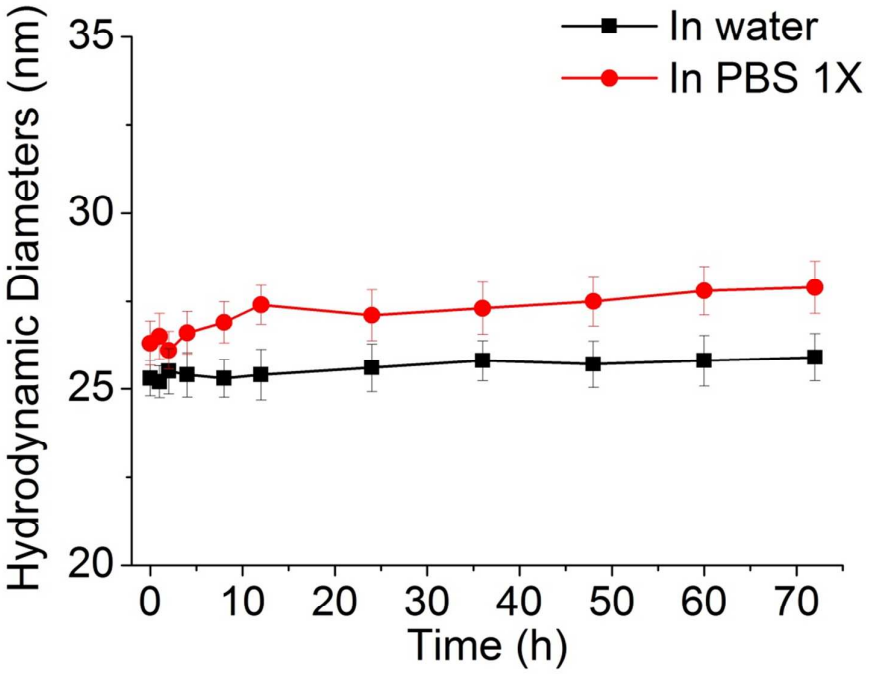

Figure S8. The temporal evolution of the hydrodynamic diameter of ZCIS NMs-25 dispersed in water and PBS (1x), respectively.

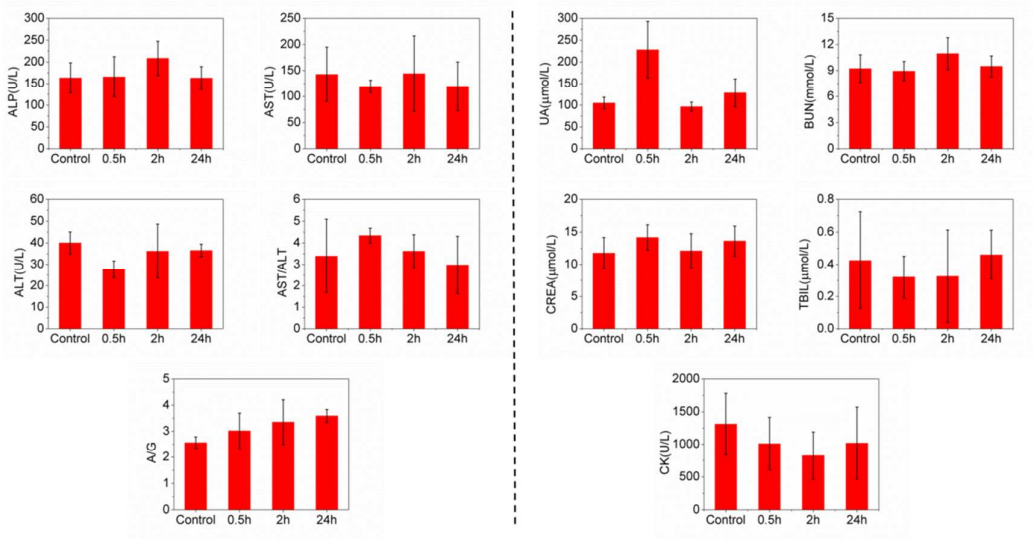

Figure S9. Blood biochemistry analysis of the mice treated with the ZCIS NMs at the different time points of 30 min, 2 h, and 24 h. The results show mean and standard deviation of aminotransferase (ALT), alkaline phosphatase (ALP), and aminotransferase (AST), total bilirubin (TBIL), blood urea nitrogen (BUN), uric acid (UA), creatinine (CREA), creatine kinase (CK).