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Supplementary Information

Compact chelator-Free Ni-integrated CuS nanoparticles with tunable near-infrared absorption and enhanced relaxivity for *in vivo* dual-modal photoacoustic/MR imaging

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Experimental Section

Synthesis of Ni-Integrated CuS NPs: Chelator-free Ni-integrated CuS NPs were prepared via high temperature one-step reaction in nonpolar solvents. A sulfur solution was first prepared by dissolving 32 mg of sulfur shot in 4 mL of oleylamine, yielding a red color solution. 0.137 g of $CuCl_2 \cdot 2.5H_2O$, 0.026 g of $NiCl_2$, 0.5g of dodecylamine and 1.5 mL of oleylamine was heated to 60 °C and was degassed under a vacuum of 20 Pa for 30 min. The temperature was then increased to 100 °C and degassed for another 30 min. The flask was then filled with argon gas, and its temperature was increased to 190 °C. The precursor Cu and Ni were dissolved completely in the solvent during the temperature increasement, and then the temperature was allowed to stabilize for several minutes. The sulfur precursor solution was rapidly injected into the premixed Cu and Ni solution at 190 °C. Aliquots of the reaction mixture were taken and quenched in cold (25 °C) chloroform. The as-prepared $Cu_{1-x}Ni_xS$ NPs were isolated through precipitation from excess ethanol, and then dissolved in chloroform.

Production of Multidentate Polymer Ligand: Imidazole-based multidentate polymer was synthesized according previously reported method. In a typical procedure, 0.1 g of poly(maleic anhydride) (MW = 5000 Da) was dispersed in 5 mL of DMSO containing 0.02 g of DMAP, 0.12 g of histamine and PEG-NH_2 was added as solid.

With the reaction going on at room temperature, the histamine and PEG-NH_2 solid gradually dissolved within 2 h. After another 4 h, the product, named as PMAH-PEG, was purified by precipitation in water followed by lyophilization. The obtained product has excellent solubility in strongly polar solvents (such as dimethyl sulfoxide, N, N-Dimethylformamide or water).

Synthesis of PMAH-PEG-capped Ni-doped CuS NPs: The Ni-integrated CuS NPs were transfer into water by ligand exchange. In a typical procedure, organic-soluble NPs were dispersed into chloroform. The NPs solution was mixed with PMAH-PEG solution of dimethyl sulfoxide, and stirred for 10 min at RT, after which tetramethylammonium hydroxide was added and stirred for an additional 20 min to form a biphasic system. The solution was centrifuged at 5000 g for 10 min to achieve phase separation. The organic layer was removed by pipette and any residual chloroform was removed from the aqueous phase by evaporation with stirring under reduced pressure. The PMAH-PEG coated NPs were precipitated by centrifugation twice with the addition of acetone and redispersed in PBS buffer at pH 7.4. Free unbound PMAH-PEG ligands were removed by an ultrafiltration device with a cutoff of 50 kDa (Millipore). For Cy5-labeled Ni-doped CuS NPs, as-prepared hydrophilic nanoparticles were reacted with Cy5-amine (Lumiprobe Corporation) using standard EDC/NHS conjugation protocol and purified with dialysis.

Ni-integrated CuS NPs Structural and Compositional Characterization: Optical absorption spectra were obtained using a PerkinElmer Lambda 750 UV–vis–nir spectrophotometer. Transmission electron microscopy (TEM) and High-resolution Transmission electron microscopy (HRTEM) images were taken on a FEI Tecnai G20 transmission microscope at 200 kV. Samples for TEM observation were prepared through dropped 10 μL of the solution on a carbon-coated gold grid and allowed the sample to dry. X-ray diffraction patterns were acquired using a Philips X'Pert diffractometer with a Cu Kα source. Inductively coupled plasma optimal emission spectrometry (ICP-OES) was used to measure the contents of the NPs.

In vitro MR imaging: The relaxation times at low field strength were measured on a 0.55 T MRI instrument (MicroMRI, Shanghai Niumag Corp.) at 32 °C. A quadrature coil with an inner diameter of 1.8 cm was used for RF transmission and reception. For T1 measurements, samples with various Ni^{2+} concentrations (determined by ICP-OES)

were dispersed in water. T1 measurements were performed using an inversion recovery (IR) sequence with various inversion times (TI). The experimental parameters were as follows: FOVRead = 80 mm, FOVPhase = 80 mm, TR = 200 ms, TE = 10 ms, Slice Width = 6.0 mm, Slice Gap = 0.5 mm.

Cell culture: MCF-7 cells (human breast cancer cells) were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, and 1% (v/v) streptomycin. Cells were incubated in a humidified incubator at 37 ℃ with 5% CO_2.

In vitro cytotoxicity: MCF-7 cells were used to assess the cytotoxicity of PMAH-PEG-capped Ni-doped CuS NPs. The cells were incubated in the presence of PMAH-PEG-capped Ni-doped CuS NPs. Cells (5×10^3 cells/well, 100 mL) were seeded into 96-well plates and grown for 12 h. The medium which contains different doses of NPs (0 – 464 ug mL^{-1}) was used to replace the old medium. Untreated cells in growth media were used as the blank control. After 24 h incubation, the cell viability was evaluated by the MTT assay.

Celll imaging: MCF-7 cells were A549 cells were cultured in ATCC-modified Eagle's minimum essential medium (EMEM) with 10% fetal bovine serum (FBS) at 37 °C (5% CO2) and were grown in 8-well LabTek chambers (Nalgene Nunc) to 20% confluency. Twenty-four hours after seeding, cells were rinsed with the medium, and NPs solution (0.46 mg mL-1) was added. The cells were washed with fresh culture medium after incubation for 1 h. Fluorescent images were acquired on a Leica TCS SP5 laser scanning confocal microscope equipped with Argon, red HeNe, and green HeNe lasers.

In vitro PA imaging: To investigate the PA signals generated by PMAH-PEG-capped Ni-doped CuS NPs, an agarose gel phantom containing different concentrations of Ni-doped CuS NPs were fabricated. PA signals and images of the phantoms were

obtained through a home-made PA imaging system which consisted of a nanosecond pulsed OPO laser (Vibrant 355 II HE, Opotek, Carlsbad, USA), a 10 MHz focused ultrasound transducer (V315-SU, Olympus IMS, Waltham, USA), a data acquisition module (CSE1422-200MHz, GaGe, Lockport, USA), and a motorized 3D scanning stage (PSA2000-11, Zolix, Beijing, China). The 1064 nm NIR laser with a repetition rate of 10 Hz and a pulse width of 5 ns was used to induce the PA signal when diffused inside the sample. The 10-MHz focused ultrasound transducer was employed to detect the PA waves, which were then amplified and transmitted to the data acquisition module for further data processing. The motorized 3D scanning stage was used to position the imaging head as well as to scan the imaging head across the *x*-*y* plane for 3D imaging. More detailed information of the system can be found in our earlier publication. [1]

Animals: All experiments involving animals were done in accordance with protocols that approved by Institutional Animal Care and Usage Committee of Shenzhen Institutes of Advanced Technology. 6-8 weeks old female SD rats (~260g) were obtained from Guangdong Province Laboratory Animal Center (Guangzhou, China), and maintained in the institutional animal care facility.

In vivo dual-modal imaging: PMAH-PEG-capped Ni-doped CuS NPs with a concentration of 0.4 mg mL^{-1} (50ul) was injected subcutaneously into the distal part of the right anterior paw of healthy SD rat (weight: 260 g). For in vivo PA imaging, SD rat was under anaesthetic by isoflurane delivered through a nose-cone (2 % isoflurane in 100 % oxygen, gas flow rate: 2 L min^{-1}) during the whole scanning period. PA measurements of the right axillary area were carried out before and after the NPs injection. The incident laser energy density on the skin surface of the rat was about 5 mJ cm^{-2} (at a wavelength of 1064 nm), which was much lower than the "maximum permissible exposure" for human skin application regulated by ANSI at

the nanosecond pulse width and wavelength of 1064 nm (100 mJ cm^{-2}). [2] MRI experiments were performed on a clinical 3 Tesla horizontal bore magnet (SIEMENS, VERIO). The pulse sequence time parameters (TE/TR) of T1 weighted imaging were as follows: TE = 9 ms, TR = 500 ms.

Irradiation stability measurement of PMAH-PEG-capped Ni-doped CuS NPs: An experiment was conducted to test whether Ni was released from CuS nanoparticles during the PA signals generation. PMAH-PEG-capped Ni-doped CuS NPs with concentration of 0.4 mg mL^{-1} (0.1mL) dispersed in PBS was irradiated under 1064 nm NIR laser with a repetition rate of 10 Hz and a pulse width of 5 ns, which was used for in vivo PA imaging. Under irradiation for about 0.5 h, the solution was gathered by an ultrafiltration device with a cutoff of 50 kDa (Millipore). The device was centrifuged at 3000 x g for 10 min. Then the filtrate was collected and analyzed using inductively coupled plasma optimal emission spectrometry (ICP-OES) to determine the concentration of the free Ni ions in the sample. The sample in the upper of ultrafiltration device was also measured after dissolved using HNO_3 solution. Since very little (0.05 mL 0.4 mg mL-1) PMAH-PEG-capped Ni-doped CuS NPs was injected into rat. It was very difficult to detect whether Ni was released in the transport process in vivo. Therefore, an additional in vitro experiment was carried out, PMAH-PEG-capped Ni-doped CuS NPs with the same amount was dispersed in Dulbecco's Modified Eagle Medium (DMEM, HyClone) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, and 1% (v/v) streptomycin. Under the same procedure as described before, the filtrate and sample in the upper of ultrafiltration device were measured. As a control, PMAH-PEG-capped Ni-doped CuS NPs with the same amount was also measured. The results were displayed in the table. The samples demonstrated that the upper of ultrafiltration nearly had same concentration Ni element. Very little Ni element was detected by ICP-OES in the

filtrate. It indicated that there was no Ni ion released from the PMAH-PEG-capped Ni-doped CuS NPs dissolved in PBS or nutrient solution during the PA signals generation.

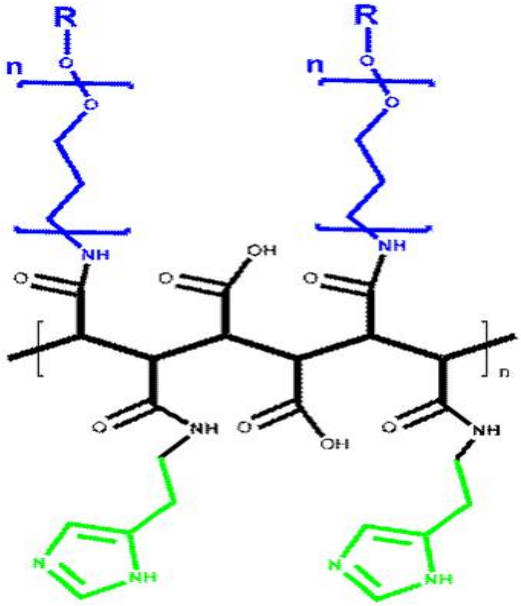

Fig.S1. Chemical structure of PMAH-PEG

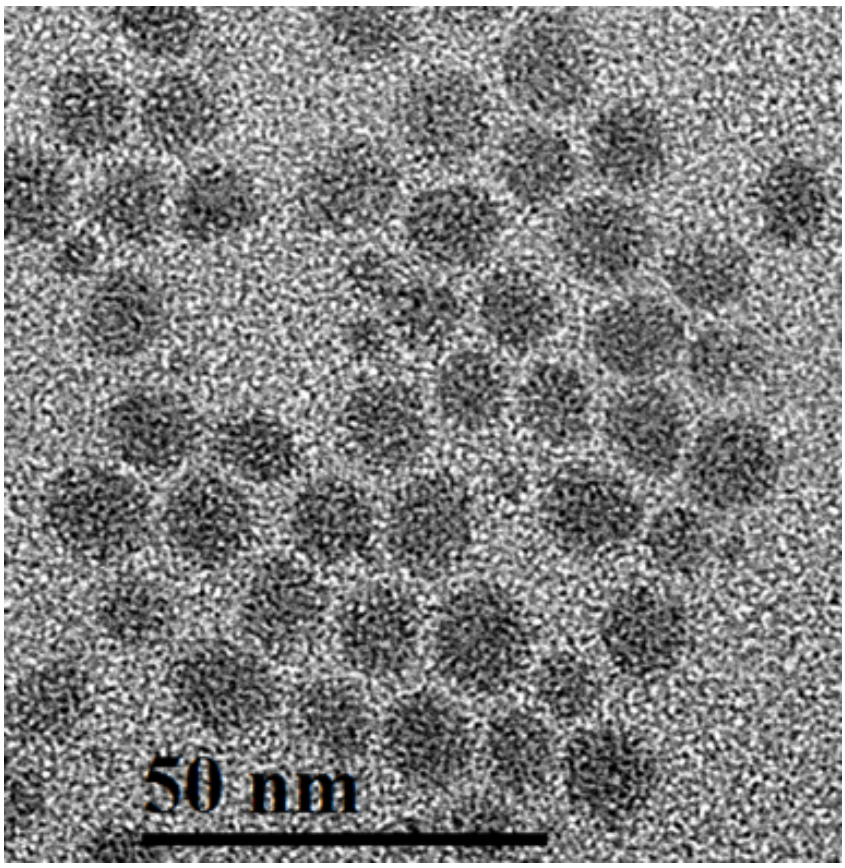

Fig. S2. Transmission electron micrograph of Ni-doped CuS NPs

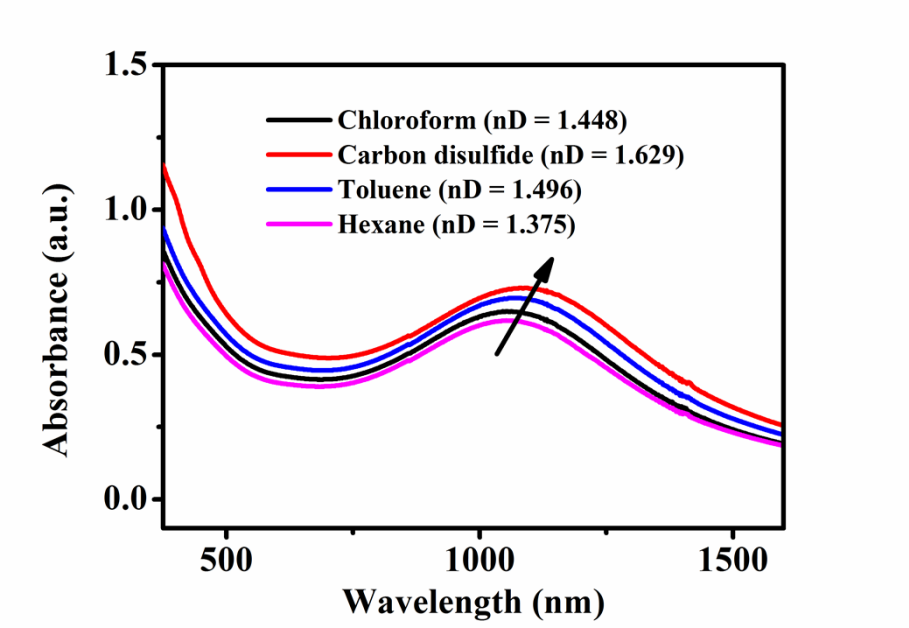

Fig.S3. UV-VIS-NIR absorption spectra of Ni-doped CuS NPs dissolved in four solvents with different refractive index

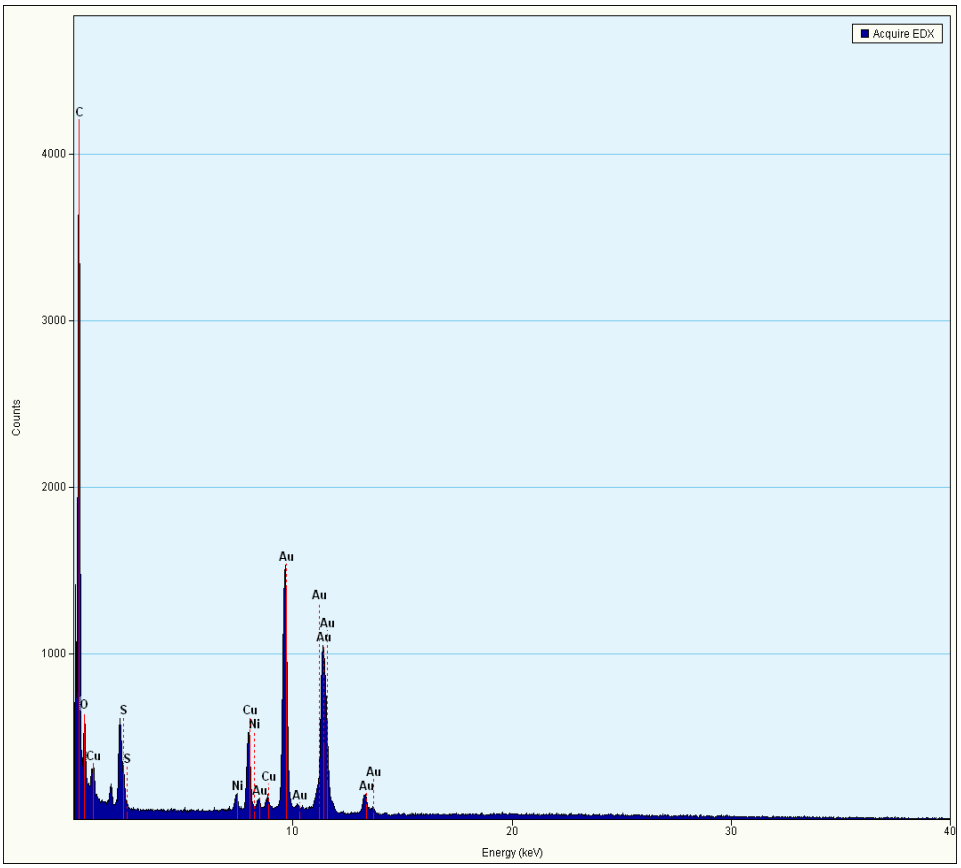

Fig.S4. EDS spectra of hydrophilic Ni-integrated CuS nanostructure

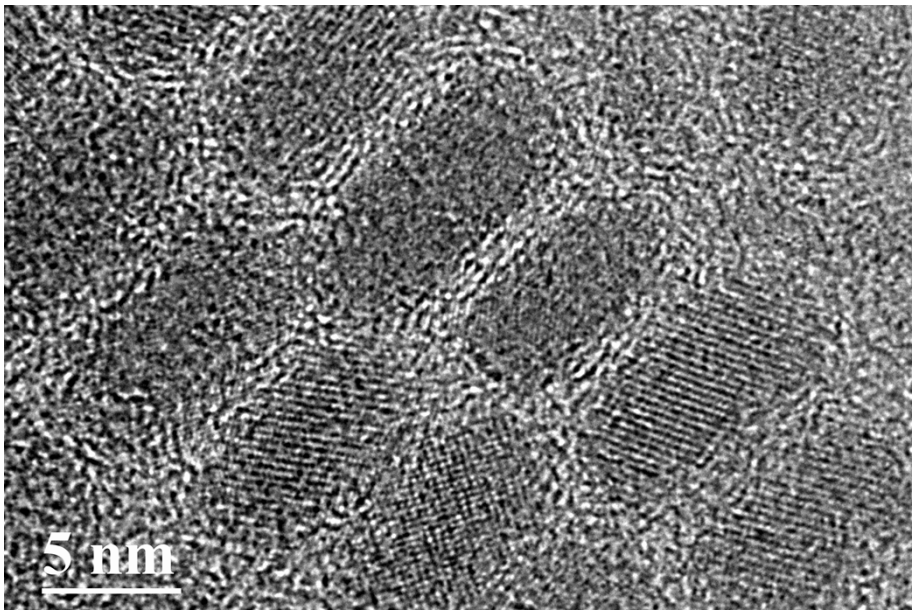

Fig. S5. High-resolution transmission electron micrograph of rod-shape Ni-doped

CuS NPs

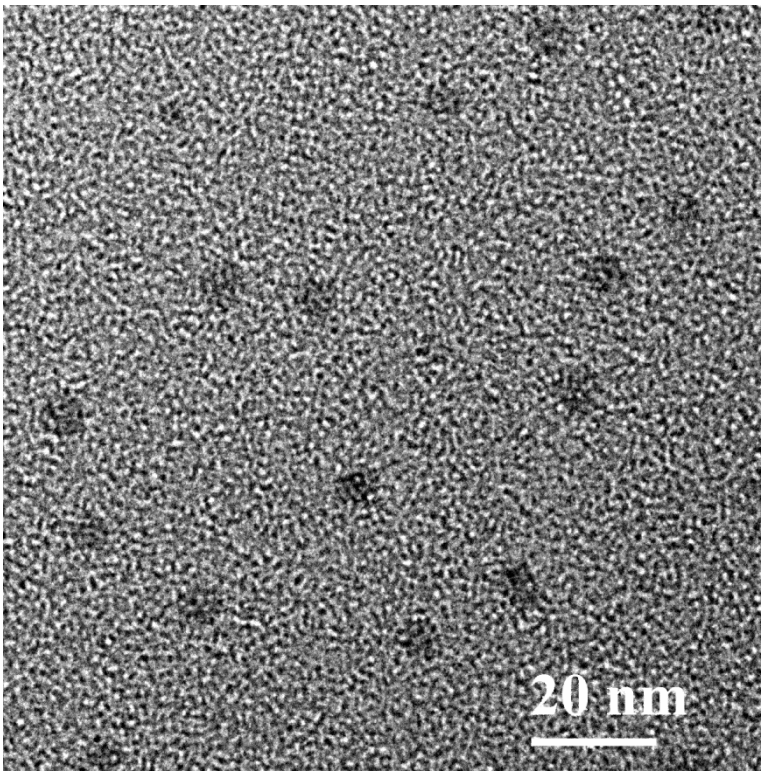

Fig. S6. TEM image of PMAH-PEG-capped Ni-integrated CuS nanoparticles

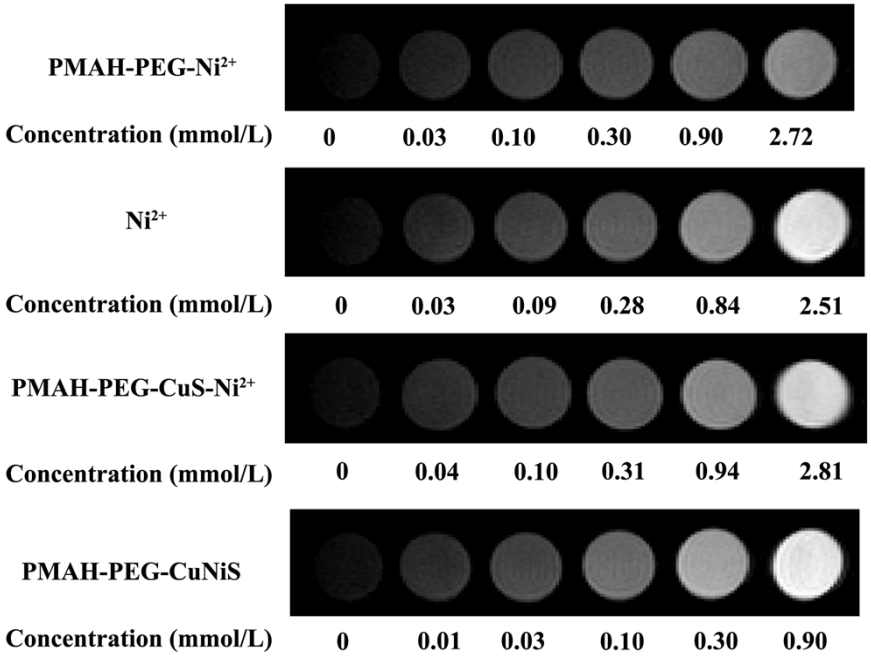

Fig. S7. T1-weighted MRI contrast images of samples at increasing Ni^{2+} concentrations

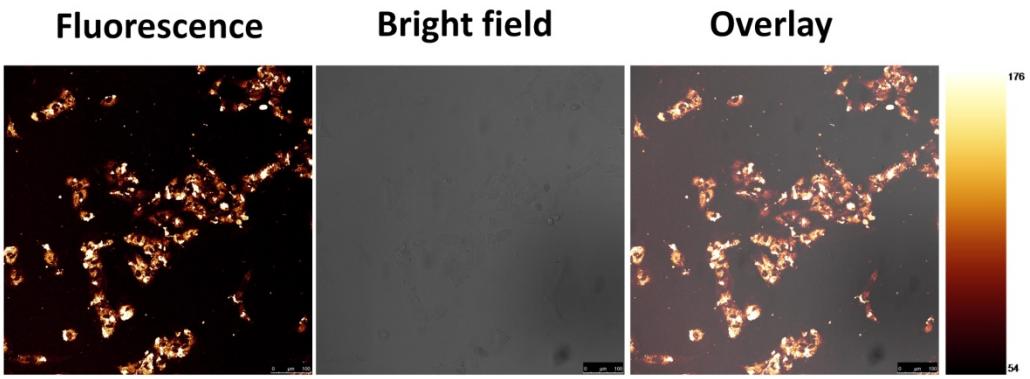

Fig. S8. Cell image experiments of PMAH-PEG-capped Ni-integrated CuS nanoparticles labeled with Cy5 dye.

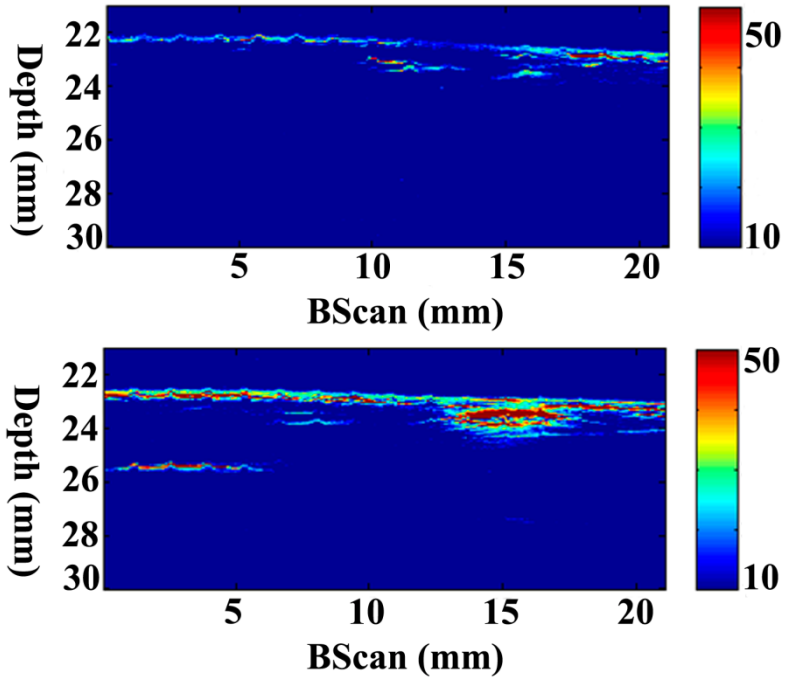

Fig. S9. PA B-scan images of the mouse before (up) and after (down) injection

contrast agents

Table S1 Measurement of Ni element by ICP-OES

Samples	Concentration of Ni (mg L^{-1}, upper)	Concentration of Ni (mg L^{-1}, filtrate)
Control	0.298	---
NPs dispersed in PBS	0.296	0.004
NPs dispersed in nutrient solution	0.301	0.002

Note: All samples were diluted to 10mL before measurement

References

[1] a) Z. H. Sheng, L. Song, J. X. Zheng, D. H. Hu, M. He, M. B. Zheng, G. H. Gao, P. Gong, P. F. Zhang, Y. F. Ma, L. T. Cai, *Biomaterials,* 2013, **34,** 5236; b) H. N. Wang, C. B. Liu, X. J. Gong, D. H. Hu, R. Q. Lin, Z. H. Sheng, C. F. Zheng, M. Yan, J. Q. Chen, L. T. Cai, L. Song, *Nanoscale,* 2014, **6,** 14270.

[2] A. N. S. Institute, ANSI Orlando: Laser Institute of America. in American National Standard for Safe Use of Lasers, American National Standards Institute Inc., New York, **2000**.