Supporting Information

Intrinsically Radioactive [^{64}Cu]CuInS/ZnS Quantum Dots for PET and Optical Imaging: improved radiochemical stability and controllable Cerenkov luminescence

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Materials.

Copper(II) chloride ($CuCl_2 \cdot 2H_2O$, 99.0%), indium chloride ($InCl_3$, 99.9%), zinc chloride ($ZnCl_2$, 99.9%), sodium hydroxide (NaOH, 97%), sodium sulfide ($Na_2S \cdot 9H_2O$, 98%) and L-glutathione (GSH, reduced) were purchased from Sigma-Aldrich. Methoxy-PEG-thiol (mPEG-SH; Mw=5000 Da) was purchased from Nanocs (New York, NY). $^{64}CuCl_2$ was produced by the PET Department, NIH. Deionized (DI) water with resistivity of 18.2 MΩ was from a Millipore Autopure system. Ethanol (99.7%) and PD-10 columns were purchased from Fisher Scientific. All of the chemicals and solvents were at least ACS-grade and used without further purification.

Preparation of stock solutions

Copper stock solution (0.02 M) was obtained by dissolving CuCl2 (0.2 mmol) in 10 ml of DI water. Indium stock solution (0.4 M) was prepared by dissolving InCl3 (1 mmol) in 2.5 ml of DI water. Glutathione (GSH) stock solution (0.1 M) was prepared by dissolving GSH (1 mmol) in 10 ml of DI water. Na2S solution (0.06 M) was freshly prepared by dissolving Na2S (0.6 mmol) in 10 ml of DI water. The zinc stock solution (0.05 M) was obtained by dissolving $ZnCl_2$ (0.5 mmol) and GSH (0.5 mmol) in 8 ml of DI water and the pH value was adjusted to 8.0 by adding 2 ml of NaOH (1 M) solution. All the stock solutions were prepared at room temperature and stored in 4 ºC for 2 weeks.

PL QYs determination

Photoluminescence QYs of CIS/ZnS QDs were determined by comparison of the integrated fluorescence intensity with Rhodamine 6G (in ethanol, PL QY=95%) with the same optical density (0.05~0.1) at the excitation wavelength (470 nm), following the equation: [1,2]

$$QY_{QDs} = 0.95 \cdot \frac{I_{QDs}}{I_{R6G}} \cdot (\frac{n_{water}}{n_{ethanol}})^2 \cdot \frac{A_{R6G}}{A_{QDs}}$$

Where I_{QDs} and I_{R6G} are the integrated fluorescence intensity of QDs and Rhodamine 6G solution, respectively; n_{water} and $n_{ethanol}$ are the refractive indexes of the solvents (water and ethanol) in which QDs and Rhodamine 6G sample are dissolved, respectively; A_{R6G} and A_{QDs} present the absorption of the Rhodamine 6G and QDs solution at 470 nm, respectively.

In vitro and in vivo CRET luminescence imaging

For the in vitro investigation, 200 μl of aqueous suspensions of different samples were placed in the 96-well black plate (Greiner Bio-One, Monroe, NC) in the light-tight chamber. All the luminescent images were acquired after 4 min scanning with various filters. For the in vivo imaging, the anesthetized mice were placed into the chamber and the images were acquired The acquired images were analyzed by the Living Image 3.0 software (Caliper Life Science, Hopkinton, MA) and the signal was normalized to photons per second per centimeter square per steradian (p/s/cm^2/sr).

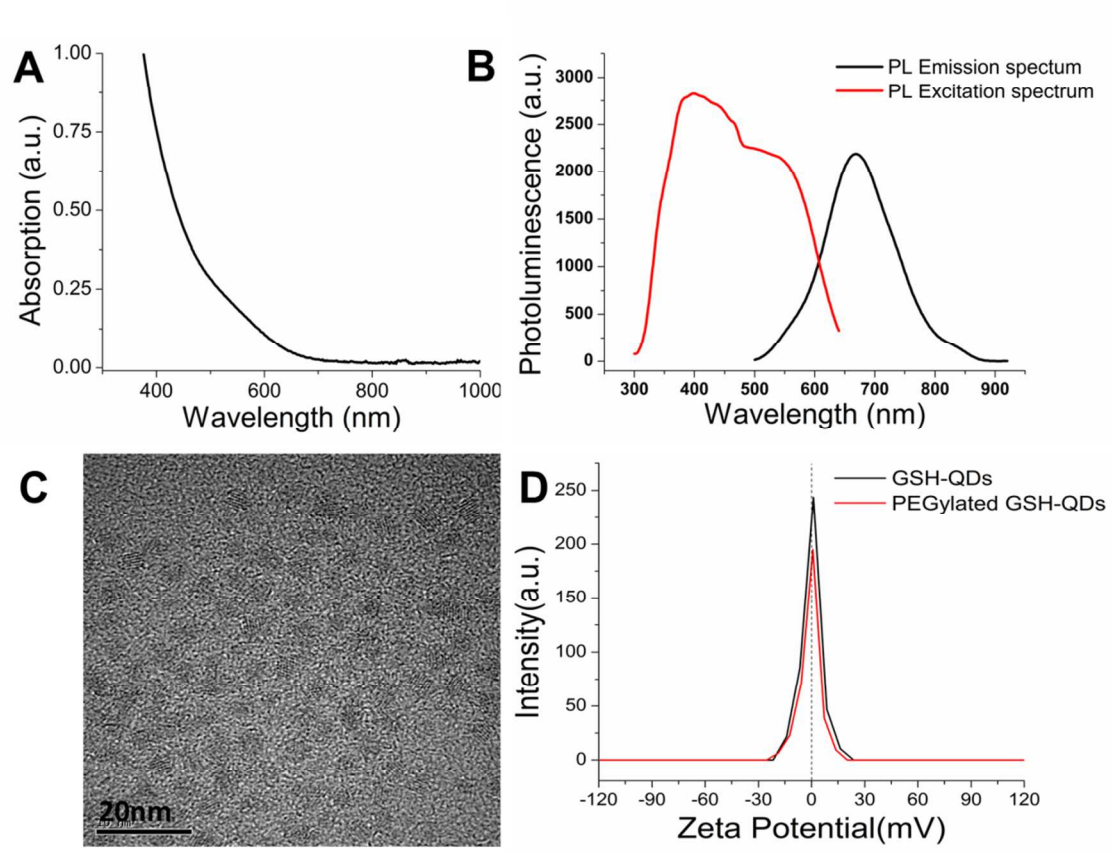

Figure S1. The absorption spectra (A), The PL emission (λ_{exc}=470 nm) and excitation spectrum (B), TEM image (C) and Zeta potential (D) of CIS/ZnS QDs.

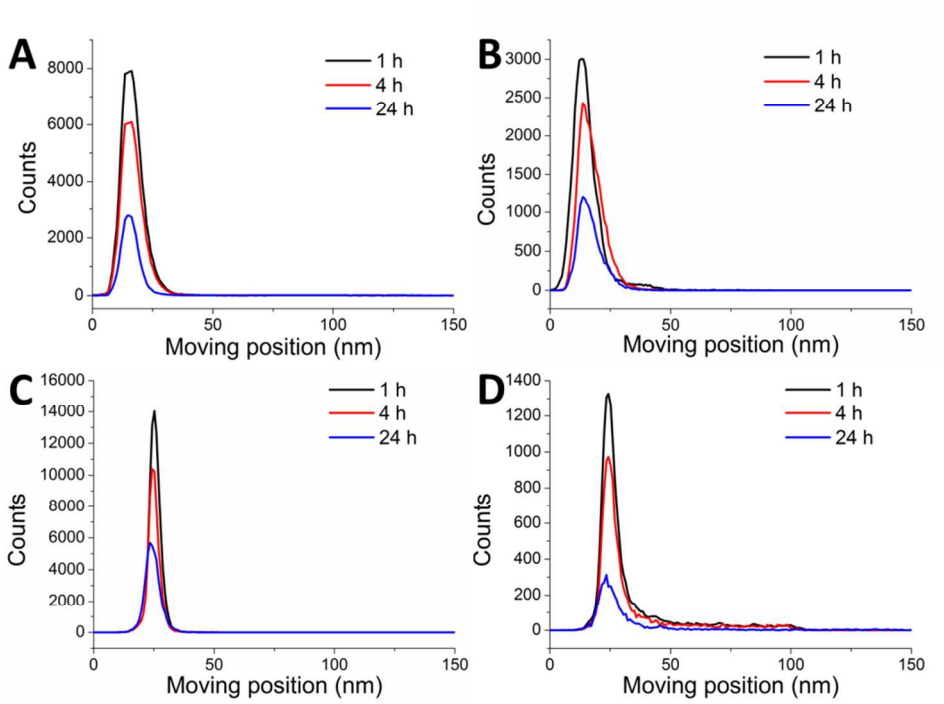

Figure S2. The radio instant thin-layer chromatography profiles of the [^{64}Cu]CIS/ZnS RQDs in PBS (A), PBS with EDTA (B), mouse serum (C) and mouse serum with EDTA (D) at 1 h, 4 h, 24 h and 48 h post incubation at 37°C

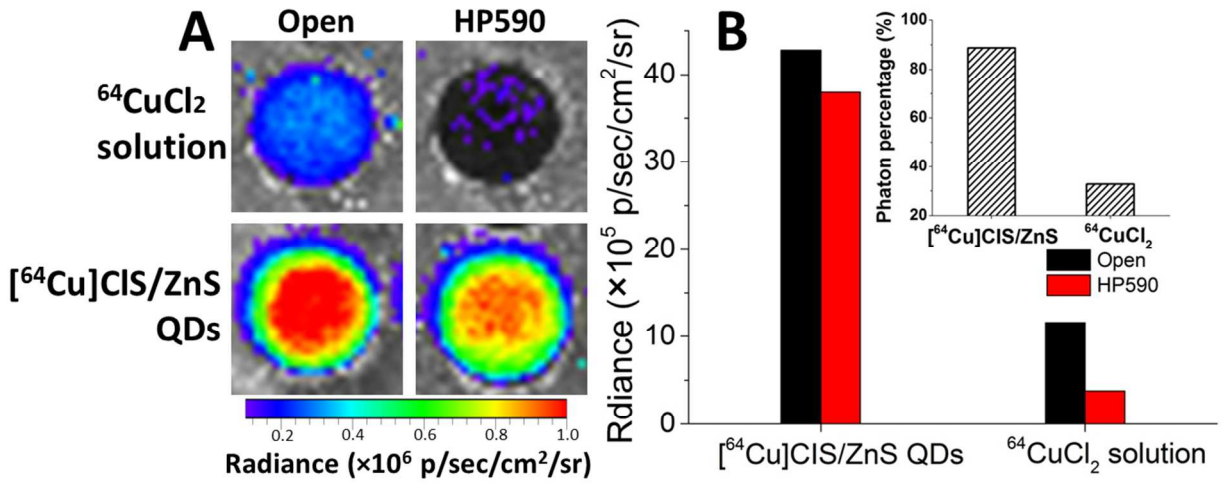

Figure S3. A, Cerenkov luminescence images of aqueous suspension of $^{64}CuCl_2$ and [^{64}Cu]CIS/ZnS RQDs with open and red filter different amount of Radioactivity and QDs in a 96-well plate. B, The corresponding photon flux with different filters. Insert, the percentage of photon flux under red filter in the total photon flux obtained with open filter. The radioactivity of the aqueous suspension was 120 μCi.

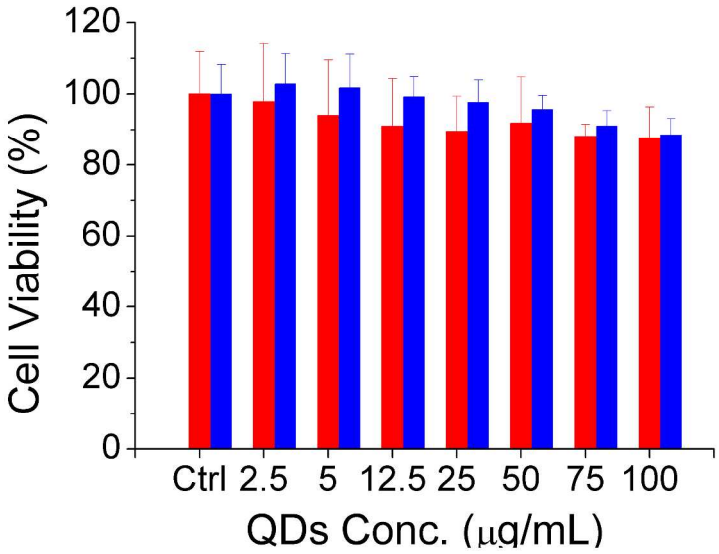

Figure S4. In vitro cytotoxicity assay on U87MG cells exposed to different concentrations of PEGylated GSH-CIS/ZnS QDs (Red) and GSH-CIS/ZnS QDs (Blue).

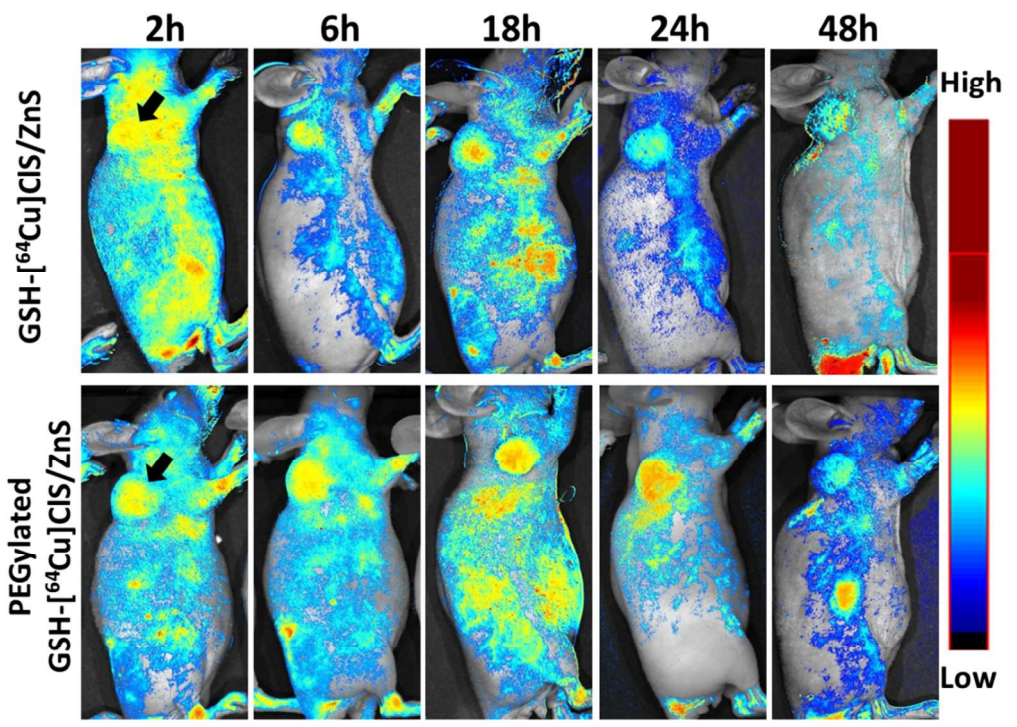

Figure S5. Representative PL images of U87MG tumor-bearing mice at 2 h, 6 h, 18 h, 24 h and 48 h after intravenous injection of 100 µl (300 µCi) of GSH-[^{64}Cu]CIS/ZnS and PEGylated GSH-[^{64}Cu]CIS/ZnS RQDs (3 mice each group). These PL images were acquired on Maestro *In Vivo* Imaging System with blue light excitation. Arrow indicates location of the tumor. (Move to SI)

Reference

1 Rurack, K.Spieles, M. Fluorescence Quantum Yields of a Series of Red and Near-Infrared Dyes Emitting at 600− 1000 nm. *Anal. Chem.* **2011**, *83*, 1232-1242.

2 Grabolle, M.; Spieles, M.; Lesnyak, V.; Gaponik, N.; Eychmüller, A.Resch-Genger, U. Determination of the Fluorescence Quantum Yield of Quantum Dots: Suitable Procedures and Achievable Uncertainties. *Anal. Chem.* **2009**, *81*, 6285-6294.