Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Long-Decay Near-Infrared-Emitting Doped-Quantum Dots for Lifetime-Based *in Vivo* pH Imaging

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1. Materials and Instrumentations

Zinc acetate ($Zn(OAc)_2$, 99.99%), glutathione (GSH) were purchased from Sigma-Aldrich. Cadmium chloride ($CdCl_2$, 99.996%), copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$, ≥99%) were purchased from Alfa Aesar. Pierce Iminobiotin Agarose Microbead (~100 μm, iminobiotin on crosslinked 6% beaded agarose, slurried in water with sodium azide) was purchased from Thermo Scientific. Water was purified with a Milli-Q water purification system. All other reagents and solvents were of analytical grade. Inductively coupled plasma optical emission spectroscopy (ICP-OES) data were taken by PerkinElmer Optima 7000 DV. The absorption spectra and fluorescence spectra were recorded on a PerkinElmer Lambda 25 absorption spectrophotometer and a FSP 920 fluorometer respectively. The samples were all excited at 400 nm using Xe-900 lamp apparatus. Transmission electron microscopy (TEM) images were taken on a FEI Tecnai G2 F20 S-Twin transmission electron microscope operating at 200 kV. X-ray diffraction (XRD) measurements were performed on Bruker D8 Advance X-ray diffractometer, which was equipped with a Cu Kα X-ray source. X-ray photoelectron spectroscopy spectra (XPS) data were taken by Thermo ESCALAB 250XI Multifunctional imaging electron spectrometer, which was equipped with a Al Kα source.

2. Synthesis of Cu-Doped Gradiently Alloyed ZnCdS QDs

$CdCl_2$ stock solution was prepared by dissolving 0.183 g $CdCl_2$ in 10 mL water. $Zn(OAc)_2$ stock solution was prepared by dissolving 0.183 g $Zn(OAc)_2$ in 10 mL

water. The GSH stabilizer solution was prepared by dissolving 0.123 g GSH in 10 mL water. $CuCl_2$ stock solution was prepared by dissolving 0.171 g $CuCl_2 \cdot 2H_2O$ in 10 mL water.

For a 10 mL synthesis of Cu-doped QDs, 20 μL of $CdCl_2$ (0.1 M), 80 μL of $Zn(OAC)_2$ (0.1 M), 0.5 mL of GSH (0.04 M) and 1 μL of $CuCl_2$ (0.1 M) were added to a 15 mL centrifuge tube and diluted to 10 mL with water, and the pH of the solution was adjusted to 10.5 by dropwise addition of 1.0 M NaOH solution while stirring. This solution was then incubated on a heat block preheated to 95 °C for 30, 60, 90 and 120 min to promote nanocrystals growth.

The original QDs were purified with an Amicon Ultra-
4 centrifugal filter device (YM-
3, Merck Millipore) via centrifugation at 8000 rpm for 20 min for several times, and t hen were redissolved in PBS buffer with the same volume.

3. Fabrication of Fluorescently Microbeads

The purified QDs-720 were selected to equipped the microbeads. Generally, 40 μL of as-prepared QDs (2.0 mg/mL) was mixed with 20 μL of microbeads. Then the fluorescently encoded microbeads were washed by water and precipitation for three times. The driving force for the incorporation process of QDs into microbeads can be attributed to the NH–O hydrogen bonding between the amino groups anchored on the surface of QDs and hydroxyl groups on the agarose miceobeads.[1]

4. Stability Measurements

The purified QDs-720 were evaluated by incubating the purified QDs in water at 37 ^{o}C, and the photoluminescence intensities were recorded at different time for a week. The photo-stabilities of QDs-720 in buffers with different pH values (6.0 and 7.0) were evaluated by continuously exciting the QDs with 400 nm laser for up to 90 min.

5. Cytotoxicity Measurements

The cytotoxicity of QDs-720 were evaluated by using the Cell Counting Kit-8 (CCK-8, Dojingdo, Kumamoto, Japan) assay. The MDA-MB-231 cells (3000 cells/well) were seeded into 96-well plates and incubated for 24 h. The cells were incubated for 24 h with various concentrations of QDs (0.1, 1, 10, 20, 50, 100, 200, and 500 μg/mL). 10 μL of CCK-8 water solution was added and further incubated for 2 h. The absorbance was measured at 450 nm with the Biotek Synergy 4 micro plate reader.

6. Lifetime-Based pH Response Experiment

Generally, 10 μL of purified QDs-720 were mixed with 90 μL of Britton-Robison buffers with different pH values (4.0, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.5, 8.0, 9.0), respectively. Then the time-resolved fluorescence decay curves of QDs-720 in buffers with different pH values were recorded on a FSP 920 fluorometer. The samples were all excited at 474 nm using laser apparatus.

7. *In Vivo* Imaging Measurement

QDs-720 were chosen for the living nude mice fluorescence lifetime imaging and NIR multicolor imaging. Eight-to-nine weeks old female Balb/c nude mice were purchase from Guangdong Province Laboratory Animal Center (Guangzhou, China), and maintained in the institutional animal care facility. The procedures were approved by Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences Animal Care and Use Committee. The nude mice were anesthetized with 1% of pentobarbital sodium (Sigma-Aldrich, USA) and 50 μl of QDs-720 were injected subcutaneously into the adjacent spots with different pH (pH= 6.0 and 7.0, respectively) on the back. The FLIM images were obtain with an time-resolved fluorescence microscopy (PicoQuant MicroTime 100, excitation, 485 nm, emission, 500 nm long-pass). And the NIR multicolour images were obtained with an *in vivo* imaging system (Maestro, CRi, Inc, excitation, 435-480 nm; emission, 490 nm long-pass).

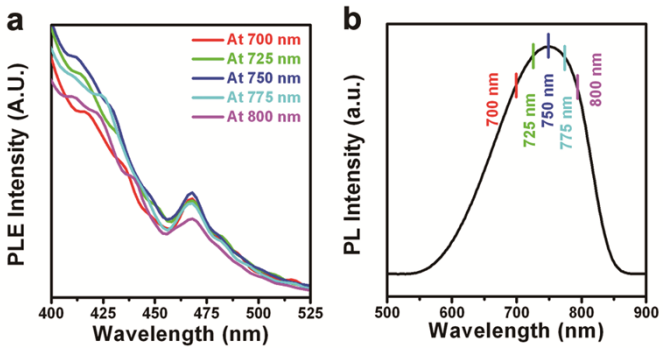

Figure S1. (a) The PLE spectra at different position of Cu-doped gradiently alloyed CdZnS QDs and (b) PL spectra of Cu-doped gradiently alloyed CdZnS QDs indicated that photoluminescence at specific stage were originated from the same set of nanocrystals.

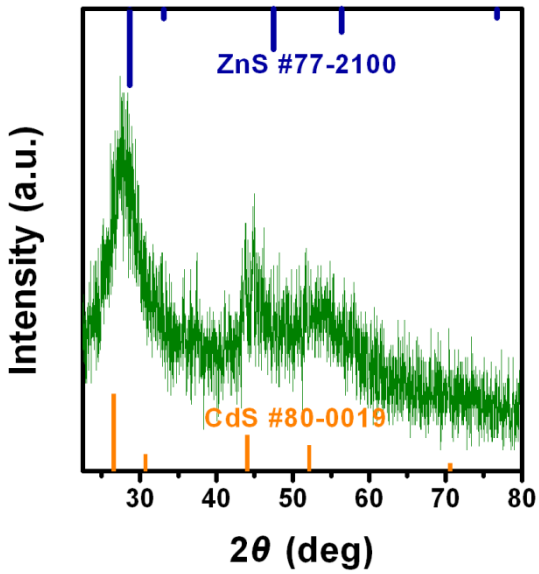

Figure S2. XRD patterns for QDs-720. Bulk diffraction peaks for zinc blende (ZB) ZnS (top) and ZB CdS (bottom) are indexed.

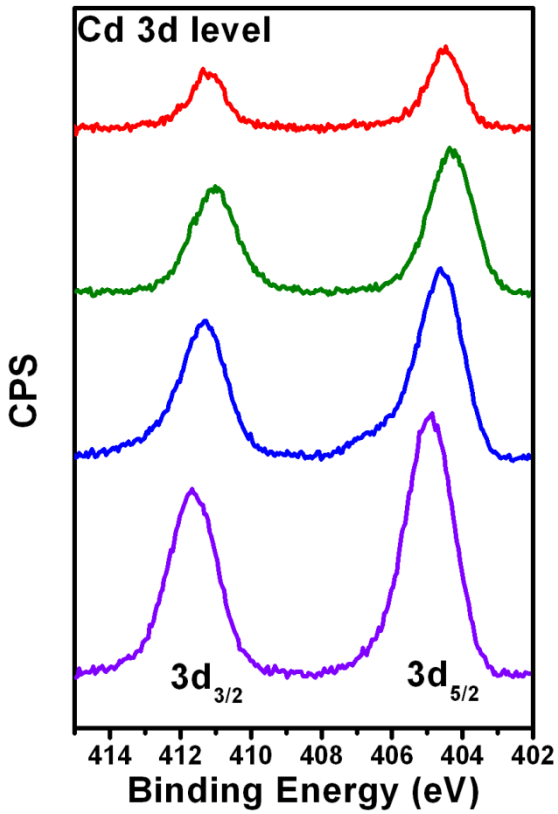

Figure S3. XPS data of the Cd 3d lever of as-prepared QDs with different heating time (from bottom to top: 30, 60, 90, and 120 min).

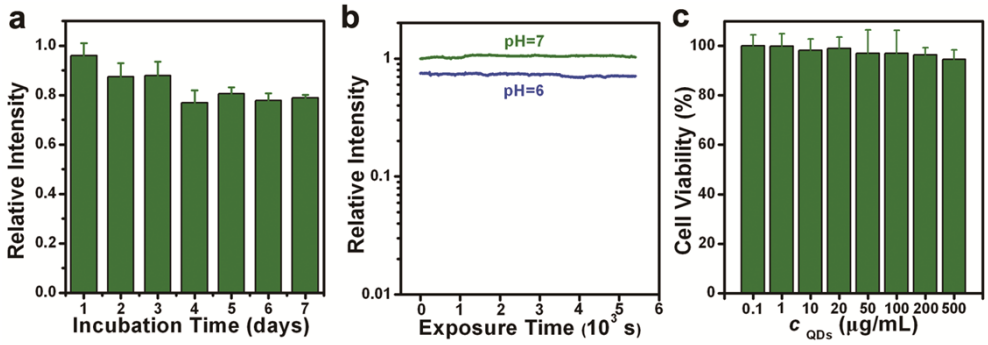

Figure S4. Stability measurements, cytotoxicity measurements experiments of QDs-720 (a) Colloidal stabilities of QDs-720 in water at 37 °C for a week. (b) Photo-stabilities of QDs-720 in buffers with different pH values (pH=6.0 and 7.0) for up to 90 min. (c) Cell viability data of MDA-MB-231 cells incubated with QDs-720 after 24 hours in various concentration (0.1, 1, 10, 20, 50, 100, 200, and 500 μg/mL).

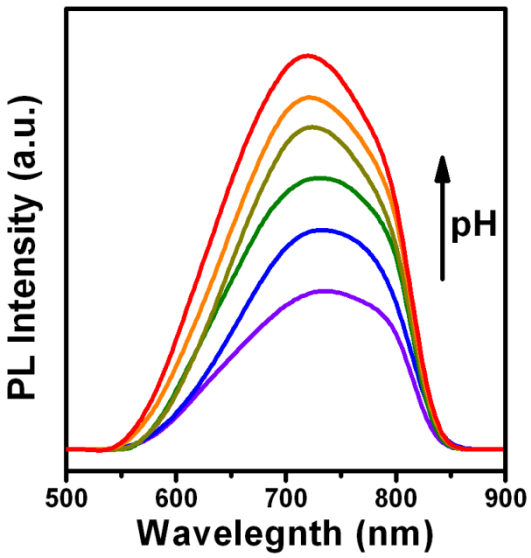

Figure S5. Fluorescence emission spectra of QD-720 in buffers with different pH values: 4.0, 5.5, 5.0, 6.0, 6.5, 7.0, and 9.0.

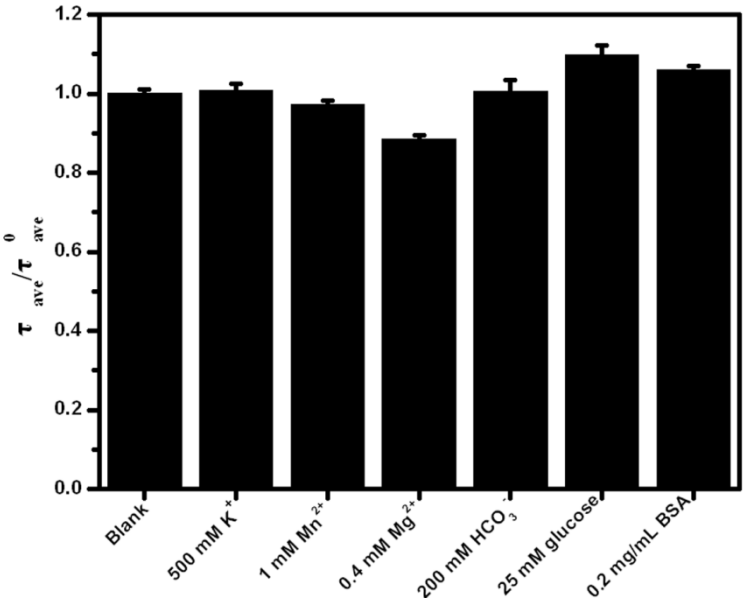

Figure S6. Interference study of the QD-720 as pH nanosensors at pH 7.0.

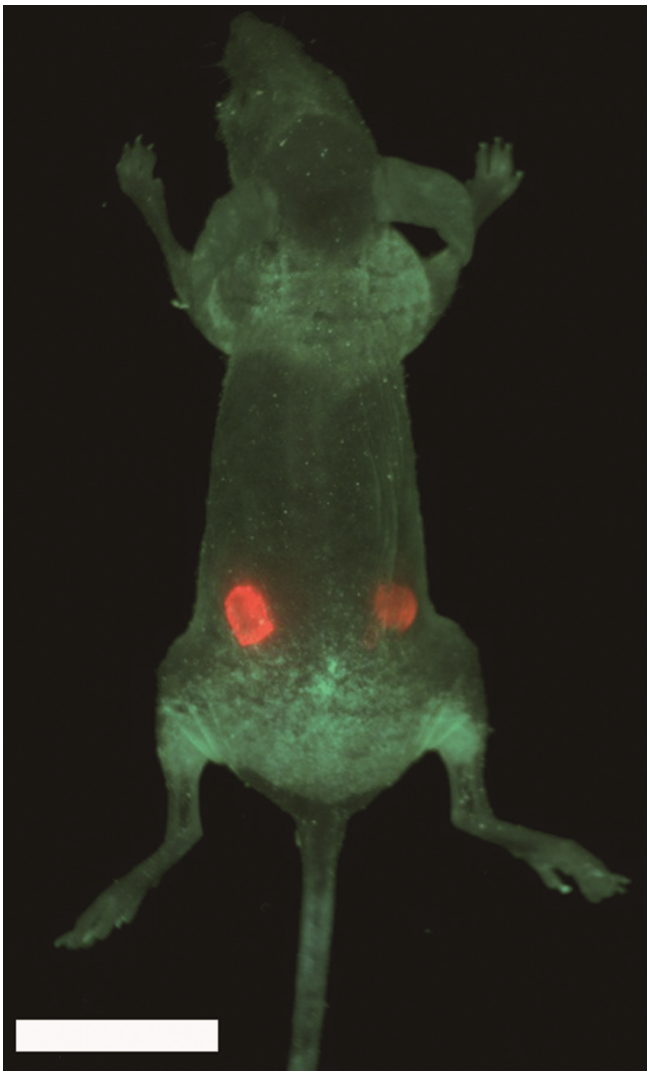

Figure S7. *In vivo* imaging experiments of QDs-720 in buffers with different pH values (scale bar: 20 cm)

Table S1. Nominal Zn/Cd and Cu/ (Zn+Cd) molar ratios used in the synthesis and the real molar ratios in Cu-doped gradiently alloyed CdZnS QDs with various reaction time determined by ICP-OES.

Reaction Time (min)	Nominal Zn/Cd molar ratios	Nominal Cu/(Zn+Cd) molar ratios	ICP results of Zn/Cd molar ratios	ICP results of Cu/ (Zn+Cd) molar ratios
30	4:1	1.00%	1.27:1	1.14%
60	4:1	1.00%	2.36:1	1.07%
90	4:1	1.00%	3.85:1	0.70%
120	4:1	1.00%	7.55:1	0.25%

Table S2. Details fitted parameter and average lifetime values of as-prepared QDs samples with different heating time.

Heating Time	a_1 (%)	τ_1 (ns)	a_2 (%)	τ_2 (ns)	x^2	$\tau_{average}$ (ns)
30	21.55	182	78.45	851	1.107	814
60	21.61	210	78.39	876	1.175	835
90	17.37	181	82.63	883	1.167	854
120	22.27	211	77.73	905	1.158	860

Table S3. Details fitted parameter and average lifetime values of as-prepared QDs samples in buffers with different pH.

pH	a_1 (%)	τ_1 (ns)	a_2 (%)	τ_2 (ns)	x^2	$\tau_{average}$ (ns)
5.5	100	262			1.026	262
5.75	100	353			1.152	353
6.0	20.02	134	79.98	541	1.040	517
6.25	18.02	141	81.98	679	1.008	655
6.5	18.53	168	81.47	752	1.036	723
6.75	20.45	180	79.55	842	1.098	807
7.0	17.37	181	82.63	883	1.167	854

Reference

1 J. Liu, X. Yang, K. Wang, Q. Wang, H. Ji, C. Wu, J. Li, X. He, J. Tang and J. Huang, *J. Mater. Chem.*, 2012, **22**, 495.