Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Dually Enriched Cu:CdS@ZnS QDs with both Polyvinylpyrrolidone Twisting and SiO_2 Loading for Improved Cell Imaging

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Experimental section

Reagents

Cadmium chloride ($CdCl_2 \cdot 5H_2O$, AR), sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$, AR), sodiumhydroxide (NaOH, AR), cyclohexane (C_6H_{12}, AR) and hexyl alcohol ($C_6H_{14}O$, AR) were purchased from Chengdu Kelong Chemical Reagent Company (Chengdu, China). $Zn(Ac)_2 \cdot 4H_2O$ (AR) and $Cu(Ac)_2 \cdot H_2O$ (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd, (Shanghai, China). Mercaptosuccinic acid (MSA, 98%), (3-aminopropyl)-triethoxysilane (APTS, 99%), ammonia solution ($NH_3 \cdot H_2O$, GR 25-28%), tetraethyl orthosilicate (TEOS, 99.99%), Triton X-100 (Biochemical grade), ethanol absolute (AR), Polyvinylpyrrolidone (PVP, MW = 8000, 24000, 58000, 1300000) were purchased from Aladdin (Shanghai, China), Polyvinylpyrrolidone (PVP, MW =10000) was bought from Sigma-Aldrich, (Shanghai, China), 4',6-diamidino-2-phenylindole (DAPI) was purchased from Beyotime (Shanghai, China), Live/Dead® Viability/Cytotoxicity Kit was purchased from Invitrogen (NewYork, USA). Ultrapure water (18.2 MΩ cm) was obtained from a water purification system (PCUJ-10, Chengdu Pure Technology Co., Ltd., Chengdu, China). All chemicals were used directly without further purification.

Characterization

UV-vis absorption spectra were collected with a UV-1700 UV/Vis spectrophotometer (Shimadzu, Japan). The measurements of PL spectra were carried out on an F-7000 spectrofluorometer equipped with a plotter unit and a quartz cell (1 cm × 1 cm) (Hitachi, Japan). TEM, EDX and SAED characterization was performed

with a Tecnai G^2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., America). Samples for TEM were prepared by dropping a dilute solution of NPs onto molybdenum grids with subsequent evaporation of the solvent in air at room temperature. The average sizes of NPs were evaluated using imageJ software based on TEM images.

Synthesis of Cu:CdS@ZnS D-dots

The synthesis conditions of Cu:CdS@ZnS D-dots were optimized and showed in Fig. S1 and Fig. S2. The parameters were chosen as follows: Cu / Cd: 0.75%; MSA / Cd: 1; S/Cd: 0.8; pH: 9.0; temperature: 100℃; time: 20 min; and layer of ZnS: 2 layers.

In a typical synthesis, aqueous solutions of $CdCl_2$ (0.4 mL, 0.4 M), $Cu(Ac)_2$ (60 μL, 0.02 M), and MSA (1.0 mL, 0.32 M) were added into a four-necked flask and the final volume of the mixture was made up to 50 mL with ultrapure water. The pH of the mixed solution was adjusted to 9.0 with 2.0 M NaOH. Then, aqueous solution of Na_2S (0.32 mL, 0.4 M) was quickly injected into the reaction mixture. The mixed solution was placed in a microwave synthesizer (SINEO Uwave-1000, Shanghai Xinyi Instrument Equipments Co. Ltd., Shanghai, China) and heated at 100 ºC for 20 min with atmospheric reflux device.

Deposition of ZnS shell around the Cu:CdS core template was carried out in the crude Cu:CdS reaction mixture. The Zn precursor solution (3 mL, 2.67 mM) was injected into the reaction system at 1.0 mL portion every 5 min. During the overcoating process, the heating program and temperature were the same as those for

synthesis of Cu-doped CdS QDs.

For purification, the obtained crude solution of QDs were precipitated with ethanol, separated by centrifuging, washed with ethanol, and dried in vacuum. The prepared QDs powder is highly soluble in water.

Synthesis of NH_2-SiO_2

The SiO_2 particles were prepared by the reverse microemulsion method. Typically, cyclohexane (7.5 mL), hexyl alcohol (1.8 mL), Triton X-100 (1.77 mL), $NH_3 \cdot H_2O$ (0.24 mL), and H_2O (0.4 mL) were added to a flask under stirring. After the formation of the microemulsion system (30 min later), TEOS (0.1 mL) was introduced. The mixture was sealed and stirred in the dark for three days. To terminate the reaction, acetone was introduced and the resultant SiO_2 particles were washed sequentially with butanol, 2-propanol, ethanol and water.

For amine functionalization, the above SiO_2 particles were first dispersed in ethanol with the concentration of 20 mg/mL. Then, (3-Aminopropyl) triethoxysilane (APTS, 0.1 mL), $NH_3 \cdot H_2O$ (1 mL), and H_2O (0.3 mL) were added. The mixture was stirred for 12 h in the dark and then refluxed for 1 h. Excess reactants were removed from the NH_2-SiO_2 by repeated centrifugation in ethanol for at least three times.

Synthesis of S NPs

To a stock solution of Cu:CdS@ZnS QDs (1 mL, 12 mg/mL), 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC, 5mg) and N-hydroxysuccinimide (NHS, 2 mg) was add to activate the carboxyl groups on the

surface of QDs for 20 min. Then, aqueous dispersion of NH_2-SiO_2 (20 mg/mL) was added and the mixture was further stirred for 2 h. The resulting SiO_2@QDs were centrifuged and washed with ultrapure water for three times to collect the precipitation.

For shelling silica, the resultant SiO_2@QDs was dispersed in a mixed solution of ammonia (4.2%, V: V) and ethanol. Then, 36 μL of TEOS was added under stirring and the reaction mixture was stirred for 5 h. S-NPs was purified by repeated centrifugation and dried in vacuum.

For preparation of S-NPs of undoped CdS@ZnS QDs, the steps were the same as those for Cu:CdS@ZnS d-dots, except that the precursor QDs were undoped CdS@ZnS QDs.

Synthesis of D-NPs

For preparation of the D-NPs, both Cu:CdS@ZnS d-dots and undoped CdS@ZnS QDs were first treated with PVP. Briefly, PVP solution was prepared by dissolving 36 mg of PVP in 1 mL ultrapure water and ultrasonicated for 30 min. Subsequently, the PVP solution (1 mL, 36 mg/mL) and the solution of QDs (1 mL, 12 mg/mL) were mixed by stirring for 24 h at room temperature. The anchoring processes of PVP-QDs assembly onto the surface of NH_2-SiO_2 were the same as those for S-NPs described above.

Evaluation of the enrichment factors

The loading number of d-dots was evaluated statistically from at least 50 PVP-QDs assemblies on multiple TEM images. We first tried to figure out each PVP-QDs

assembly from high-resolution TEM image and numbered the d-dots in each assembly. Then the loading number of d-dots per PVP molecules was obtained through averaging of that from multiple PVP-QDs assemblies. For S-NPs and D-NPs, the method for obtaining the enrichment factors was similar.

Cell cytotoxicity of NPs

The cytotoxicity of NPs was evaluated with HeLa cell lines using an MTT assay. Briefly, HeLa cells were seeded in a 24-well plate at 5×10^4 cells per well. After cell attachment for 24 h, the cells were washed with PBS and incubated with 0, 25, 50, 75, 100, 125 μg/mL various NPs for 24 h. Then cells were incubated in the media containing 0.5 mg/mL MTT for 4 h, after which the medium was carefully removed. Subsequently, 450 μL DMSO was added to each well and incubated for 0.5 h. The absorbance was measured at 490 nm with a microplate reader (1420, Perkin Elmer Co. Ltd., USA). Statistical analysis was carried out using SPSS with a Tukey test. A significant difference was considered when $p < 0.05$.

Confocal imaging

HeLa cells (1×10^4 cell/mL) were seeded on clean coverslips in a 6-well plate, followed by incubation with culture media 1640 at 37 ºC for 24 h. The cells were rinsed using PBS. Then, Cu:CdS@ZnS d-dots, S-NPs, and D-NPs (both from d-dots) were set at the same concentration according to absorbance measurements (Fig. 3 in the main text, corresponding to mass concentration of 50 μg/mL of D-NPs) and ultrafiltered with a Millipore Miliex@GP ultrafilter (0.22 μm). The ultrafiltered d-dots, S-NPs and D-NPs in PBS buffer were added to the cell culture media and used

for feeding the HeLa cells for 24 h, 48 h, and 72 h, respectivley. The NPs treated HeLa cells were fixed using a polyoxymethylene solution (4%) under room temperature for 20 min. For cell co-staining, the D-NPs were first incubated with HeLa cells for 24 h and treated with Live/Dead® Viability/Cytotoxicity Kit for 30 min before fixed with polyoxymethylene solution (4%) under room temperature for 20 min. After that the fixed cells were further treated with DAPI for 10 min. Then the fixed cells were taken for cell imaging with a Nikon A1 laser scanning confocal microscope with the excitation wavelength of 405 nm.

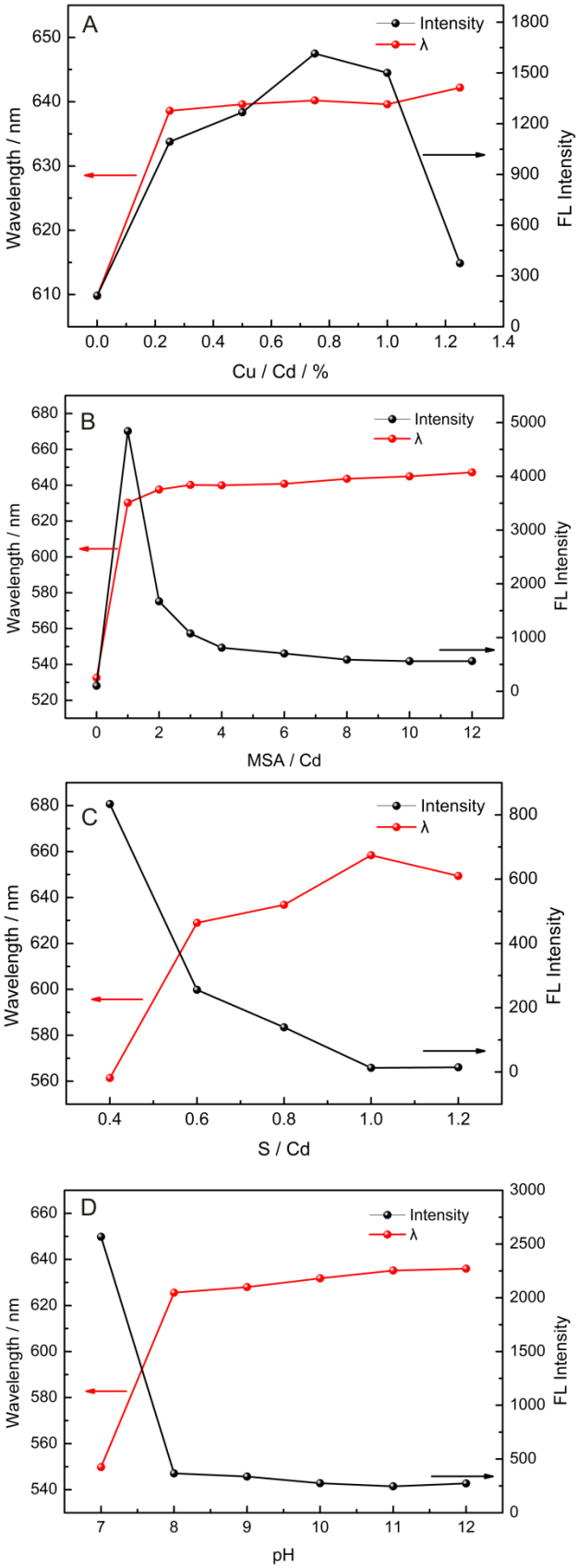

Fig. S1 Optimization of the synthetic conditions for the Cu:CdS@ZnS d-dots: (A) the precursor molar ratio of Cu^{2+} to Cd^{2+}; (B) the precursor molar ratio of MSA to Cd^{2+}; (C) the precursor molar ration of S^{2-} to Cd^{2+}; and (D) precursor pH.

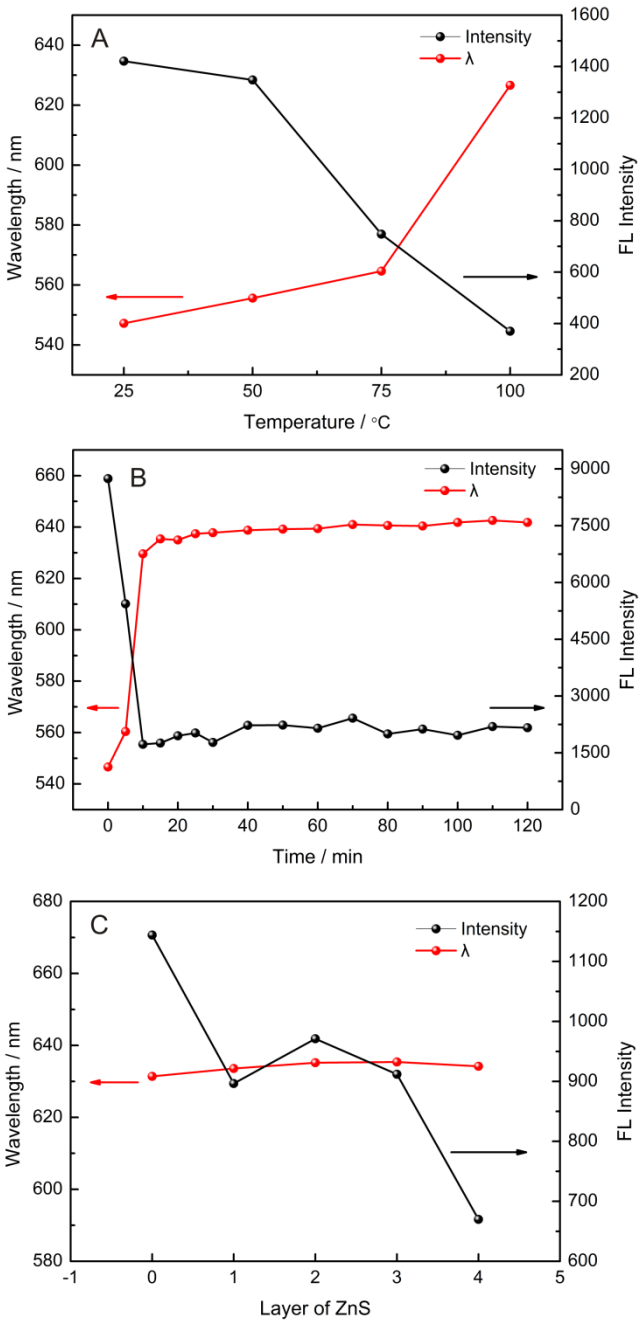

Fig. S2 Optimization of the synthetic conditions for the Cu:CdS@ZnS d-dots: (A) reflux temperature; (B) reflux time; and (C) number of ZnS layer shelled around the original d-dots.

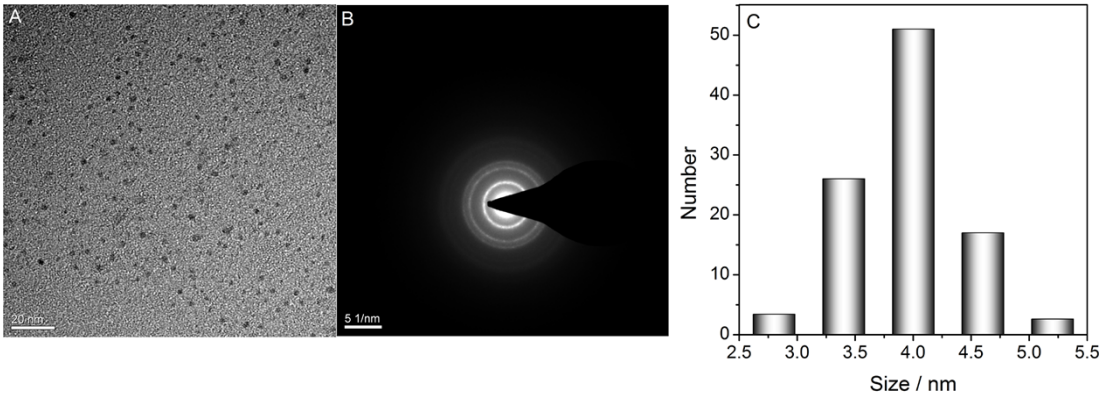

Fig. S3 (A) TEM image of d-dots with scale bar of 20 nm; (B) selected area electron diffraction (SAED) pattern of d-dots; and (C) the size distribution of d-dots.

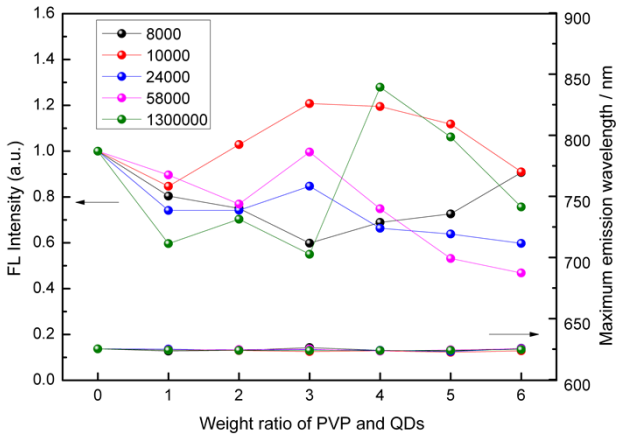

Fig. S4 Effects of PVP MWs on the emission wavelengths and emission intensities of Cu-doped CdS@ZnS QDs. Concentration of QDs: 1 mg/mL.

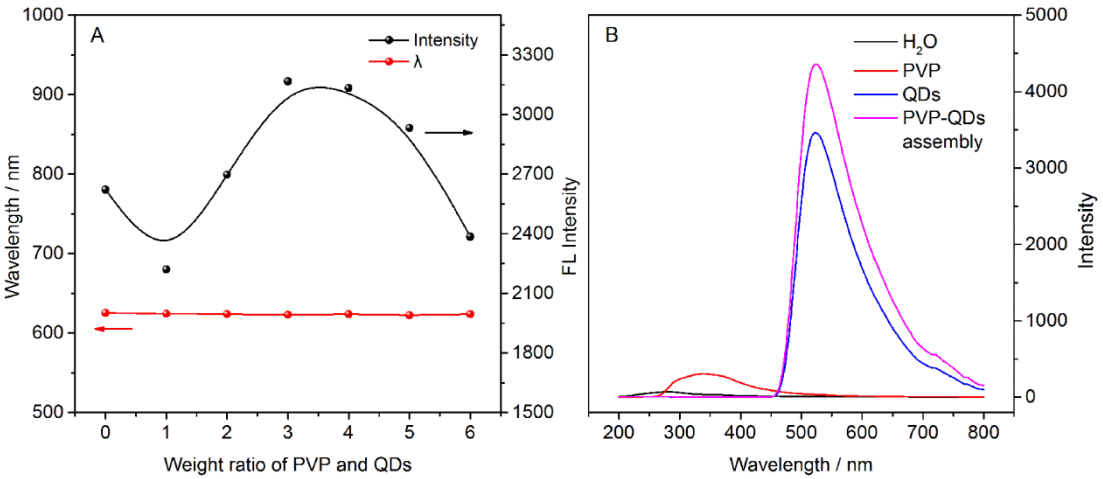

Fig. S5 The interaction between PVP (MW of 10000) and QDs: (A) the influences of PVP/QDs weight ratios on the maximum fluorescence emission wavelengths and intensities of QDs; and (B) light scattering spectra. Concentration of QDs: 1 mg/mL.

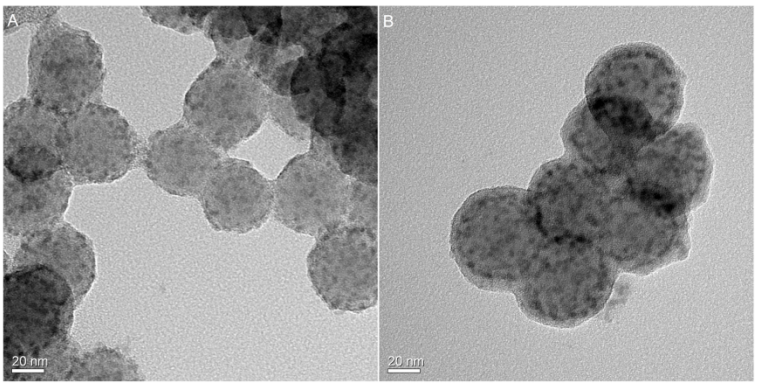

Fig. S6 TEM characterization of S-NPs: (A) SiO_2@QDs; and (B) SiO_2@QDs@SiO_2.

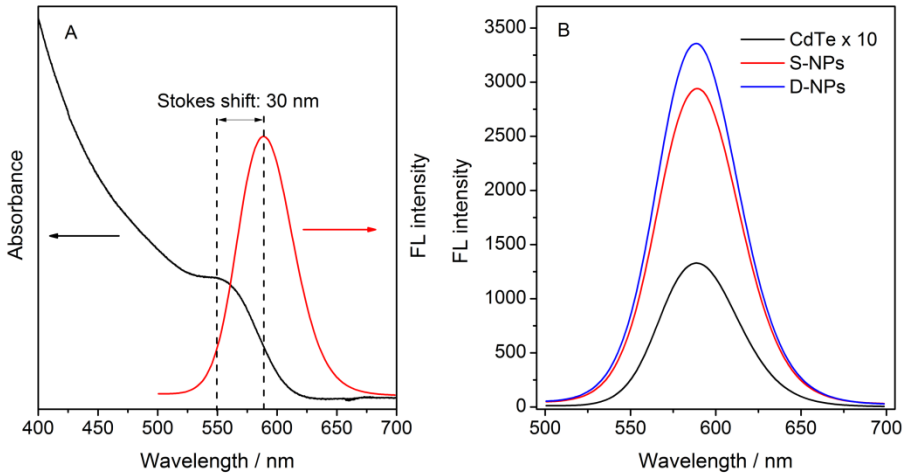

Fig. S7 (A) The UV-vis absorption and fluorescence emission spectra of CdTe QDs; and (B) Fluorescence performance of the S-NPs and D-NPs from CdTe QDs (ex: 365 nm).

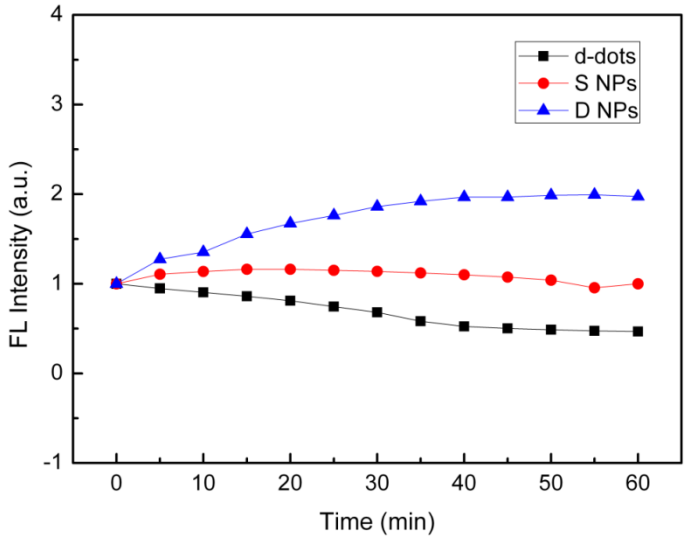

Fig. S8 The photo-stability of Cu:CdS@ZnS QDs, S-NPs and D-NPs. All the three NPs were irradiated with 365 nm UV light (slit: 10 nm; and UV power: 1.0 mW/cm^2).

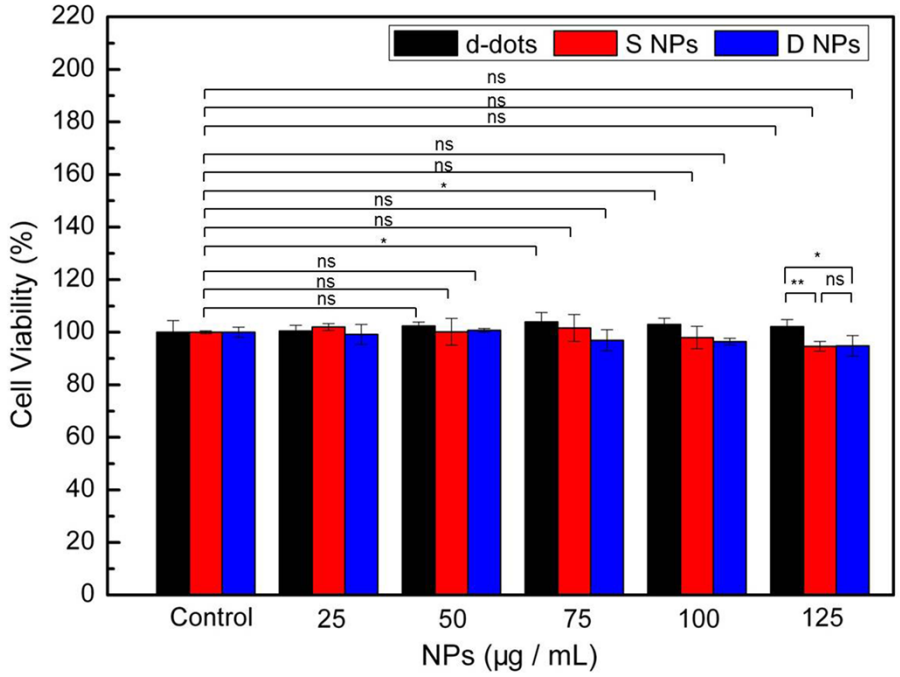

Fig. S9 The viability of HeLa cells in vitro measured by the MTT assay. (Error bars represent standard deviation from the mean (n = 3). ns (no significant difference): $p>0.05$. *: $p<0.05$. **: $p<0.01$.)

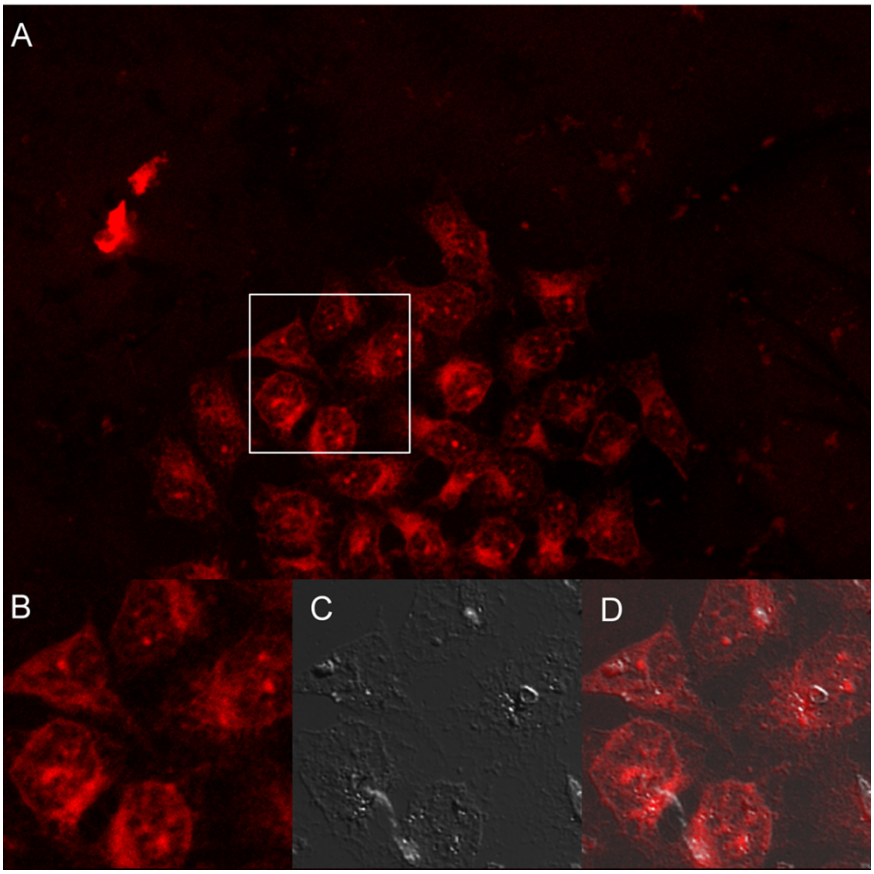

Fig. S10 (A) CLSM image of HeLa cells incubate with D-NPs for 24 h; (B) magnified image of the selected area in A; (C) the bright-field transmission image of the selected area; and (D) merged image of B and C.

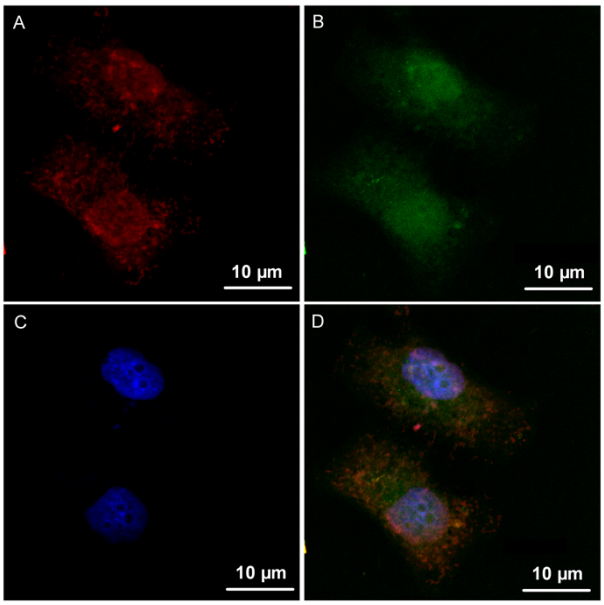

Fig. S11 The CLSM images of HeLa cells treated with D-NPs for 24 h, Live/Dead® Viability/Cytotoxicity Kit (Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely green fluorescent calcein) for 0.5 h, and DAPI (4',6-diamidino-2-phenylindole, blue, a specific dye for staining nucleus) for 10 min. The images were obtained with different filters for: (A), D-NPs fluorescence; (B), green fluorescence from calcein (Live/Dead® Viability/Cytotoxicity Kit); (C), blue DAPI fluorescence; and (D) overlay of A-C. These images confirmed the even distribution of D-NPs inside cells.

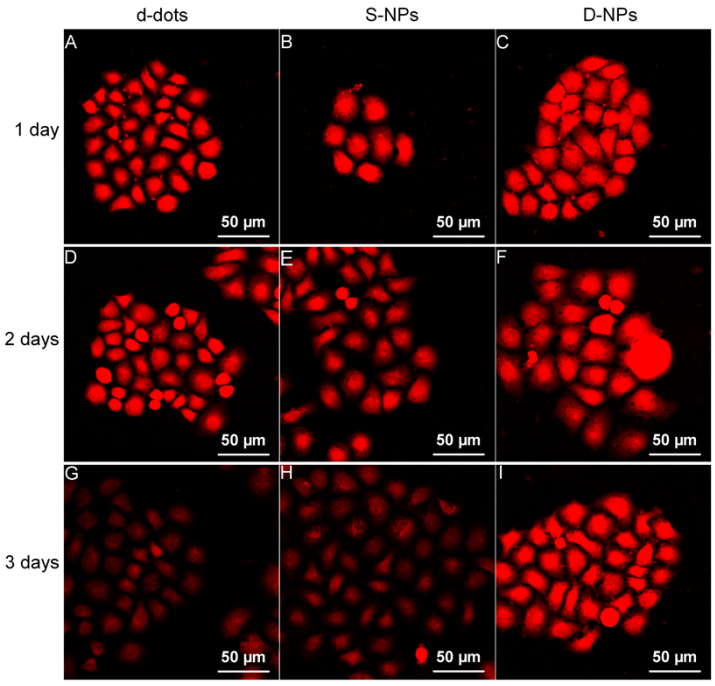

Fig. S12 CLSM images (excited with 405 nm laser) of HeLa cells incubated with d-dots, S-NPs and D-NPs from d-dots for 24 h, 48 h, and 72 h, respectively. Left: d-dots; middle: S-NPs; and right: D-NPs. Scale bar: 50 μm.

Table S1 The diameter of NPs

NPs	Diameter (nm)
Cu:CdS@ZnS QDs	3.9 ± 0.4
SiO_2 NPs	45.0 ± 2.0
S-NPs	60.7 ± 3.4
D-NPs	61.4 ± 4.1

Table S2 The operation parameters of CLSM.

Fig. 4	HV	Offset	Laser power (kW/cm^2)
TRITC-red	104	-53	15.4
Pinhole	2.6		
TD	73	-56	
Fig. S12	HV	Offset	Laser power (kW/cm^2)
TRITC-red	121	-53	15.4
Pinhole	2.6		
TD	52	-56	