Supporting Information

Purple-, Blue-, and Green-Emitting Multishell Alloyed Quantum Dots: Synthesis, Characterization, and Application for Ratiometric Extracellular pH Sensing

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Scanning Transmission Electron Microscopy (STEM) and Energy-Dispersive X-ray spectroscopy (EDX). The QD sample solution was mixed with isopropanol and methanol to flocculate the QDs, centrifuged at 3,800 rpm for 5 min, and the supernatant was discarded. The QD pellet was dissolved in chloroform, a drop of the QD suspension was deposited on Au grid (ultrathin carbon film on lacey carbon support film, 300 mesh, Ted Pella, Inc.), and the QD sample was dried. Scanning transmission electron microscope (STEM) images and energy dispersive X-ray (EDX) spectra were acquired using a JEOL 2100 FEG-TEM. STEM images were obtained with Z-contrast annular dark-field imaging technique. While the EDX spectra for large area (50-100 nm rectangle) were collected for 3 minutes, those for selected spots (0.5 nm probe size) were acquired for 5-10 seconds to minimize error due to the image drift during measurement. The averaged molar ratios of each element (S, Zn, Se, Cd) with standard deviations were obtained from either large area or the randomly chosen three to five different spots (center or edge of a single QD) in each sample.

Scanning Electron Microscopy (SEM). A Hitachi SU-70 Schottky field emission gun scanning electron microscopy was used for ultra-high resolution morphological imaging and relatively fast microchemical analysis for ZnSe QDs. The ZnSe QD sample solution was mixed with isopropanol and methanol to flocculate the QDs, centrifuged at 3,800 rpm for 5 min, and the supernatant was discarded. The QD pellet was dissolved in chloroform, a drop of the QD suspension was deposited on Cu grid (ultrathin carbon film on lacey carbon support film, 400 mesh, Ted Pella, Inc.), and the QD sample was dried. The EDX spectrum using the system equipped with SEM were collected from the selected area for about 3 minutes.

Ligand Exchange. Typical procedures for preparing a ligand for ligand exchange and the subsequent ligand exchange reaction are described.

(A) DHLA-PEG750-OMe

QDs coated with native hydrophobic ligands (~5.0 nmol in stock solution) were flocculated by mixing with isopropanol and methanol. The mixture was centrifuged at 3800 rpm for 5 min. The clear supernatant was discarded. The QD pellet was mixed with 2-(2-aminoethoxyl)ethanol (0.40 ml), $CHCl_3$ (0.40 ml) and methanol (0.40 ml). The reaction mixture was stirred at 55 °C for 4.5 h under N_2. After cooling, excess ethyl acetate was added to the mixture to flocculate the QDs. The mixture was centrifuged at 3800 rpm for 5 min, and the supernatant was discarded. The QD pellet was dissolved in 0.5 ml of methanol. During the intermediate ligand exchange process, DHLA-

PEG750-OMe was premetalated with zinc complex. DHLA-PEG750-OMe (0.202 g, 2.18×10^{-4} mol), NaOH (17.5 mg, 4.38×10^{-4} mol), $Zn(NO_3)_2\cdot6H_2O$ (31.0 mg, 1.04×10^{-4} mol) and MeOH (1.0 ml) were mixed in a vial, and the mixture was stirred at 50 °C for 2 h under N_2. The ligand solution was injected by a syringe to the QD solution in methanol prepared above. The reaction mixture was stirred at 45 °C overnight under N_2. After cooling, the QDs were flocculated by a mixture of ethyl acetate and hexane. The mixture was centrifuged at 3800 rpm for 5 min, and the supernatant was discarded. The residual solvent was evaporated, and the QD pellet was dissolved in deionized (DI) water (~2 ml). The QD solution was filtered through a Millex-LCR membrane filter (pore size 0.45 μm, Millipore) and transferred to a centrifugal spin dialyzer (Amicon Ultra 50K, Millipore). The mixture was diluted with DI water and centrifuged at 3800 rpm for 5~10 min, and the clear, filtered solution was discarded. To remove excess unbound ligands and other byproducts, the QD dispersion was subject to a few additional rounds of centrifugation with DI water, followed by filtration through a Millex-LG membrane filter (pore size 0.20 μm, Millipore).

(B) CL4

QDs coated with native hydrophobic ligands (~6.0 nmol in stock solution) were flocculated by mixing with isopropanol and methanol. The mixture was centrifuged at 3800 rpm for 5 min. The clear supernatant was discarded. The QD pellet was dissolved in 1.0 ml of $CHCl_3$. For the ligand preparation, LiOH (21.5 mg, 8.98×10^{-4} mol) was added to a mixture of CL4 methyl ester precursor (0.153 g, 3.64×10^{-4} mol),[1] methanol (0.8 ml) and DI water (1.0 ml). The reaction mixture was stirred at room temperature for 1 h. 4 M HCl was then added dropwise to the reaction mixture to adjust the pH to~7, and $NaBH_4$ (29.5 mg, 7.80×10^{-4} mol) was added to the aqueous solution, which was further stirred at room temperature for 1 h under N_2. Then, 4 M HCl was added dropwise to the reaction mixture to adjust the pH to ~7. The ligand solution was injected by a syringe into the QD solution in $CHCl_3$ with vigorous stirring. The biphasic mixture was stirred at 47 °C overnight under N_2. After cooling, the $CHCl_3$ layer was collected by a syringe and discarded. The residual $CHCl_3$ in the aqueous layer was removed by evaporation. The aqueous layer was then filtered through a Millex-LCR membrane filter (pore size 0.45 μm, Millipore) and transferred to a centrifugal spin dialyzer (Amicon Ultra 50K, Millipore). The mixture was diluted with DI water and centrifuged at 3800 rpm for 5~10 min, and the clear, filtered solution was discarded. To remove excess unbound ligands and other byproducts, the QD dispersion was subject to a few

additional rounds of centrifugation with DI water, followed by filtration through a Millex-LG membrane filter (pore size 0.20 μm, Millipore). Ligand exchange with CL2 was carried out in a similar manner.

pH Stability Test. QD samples were dissolved in buffer solutions of different pH, and the QD concentrations were adjusted to ~0.5 μM. Buffer solutions used are as follows: 50 mM KCl + HCl for pH 2; 0.1 M AcOH + 0.1 M NaOAc for pH 3-6; 50 mM Tris + HCl for pH 7 and 9; 25 mM $NaHCO_3$ + NaOH for pH 11; 50 mM KCl + NaOH for pH 12 and 13. All samples were stored in a refrigerator, and the photographs were periodically collected.

Salt Stability Test. QD samples were dissolved in concentrated NaCl solutions, and the QD and NaCl concentrations were adjusted to ~0.5 μM and 3 M, respectively. All samples were stored in a refrigerator, and the photographs were periodically collected.

FRET Analysis. FRET efficiency E_n was determined using:

$$E_n = \frac{F_D - F_{DA}}{F_D} \qquad \text{(Eq. 1)}$$

where n is the ratio or valence of dye-acceptors per QD and F_D, F_{DA} designate the fluorescence intensities of the QD donor alone and the donor in the presence of acceptor(s), respectively.[2] Förster distance (R_0) corresponds to a FRET efficiency E_n of 50% for a configuration consisting of a single QD donor coupled to a single dye-acceptor and is defined by:[2]

$$R_0 = \left(\frac{9000(\ln 10)\kappa^2 Q_D}{128\pi^5 \breve{n}^4 N_A} J\right)^{1/6} \qquad \text{(Eq. 2)}$$

where $\breve{n}$ is the refractive index of the buffer medium, N_A is Avogadro's number, Q_D is the fluorescence quantum yield (QY) of the donor, J is the spectral overlap integral, and κ^2 is the dipole orientation factor. We use a κ^2 of 2/3 which is appropriate for the random dipole orientations found within these self-assembled configurations as described.[3]

Characterization of the QD-FITC Conjugates Using Agarose Gel Electrophoresis. 464 nm emitting QDs coated with DHLA-PEG750-OMe:DHLA-PEG600-NH_2 (4:1) (1.0 μM), the QD-FITC conjugates (1.0 μM) and FITC (10 μM) were prepared in 1× PBS solution. 5 pmol of the unconjugated QDs, 5 pmol of the QD-FITC conjugates and 250 pmol of FITC were utilized. Each sample was mixed with 30% glycerol loading buffer, loaded in a well and then run on 0.8 %

agarose gel using 1× TBE buffer (90 mM Tris borate, 2mM EDTA, pH 8.3) for ~30 min with a field strength of ~12 V/cm gel length. Fluorescent gel images were collected on a BioRad Molecular Imager ChemiDoc XRS+ system using UV excitation.

References

(1) Susumu, K.; Oh, E.; Delehanty, J. B.; Blanco-Canosa, J. B.; Johnson, B. J.; Jain, V.; Hervey, W. J.; Algar, W. R.; Boeneman, K.; Dawson, P. E.; Medintz, I. L. *J. Am. Chem. Soc.* **2011**, *133*, 9480.

(2) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; 3rd ed.; Springer: New York, 2006.

(3) Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.; Bawendi, M. G.; Mattoussi, H. *J. Am. Chem. Soc.* **2004**, *126*, 301.

Supporting Figures:

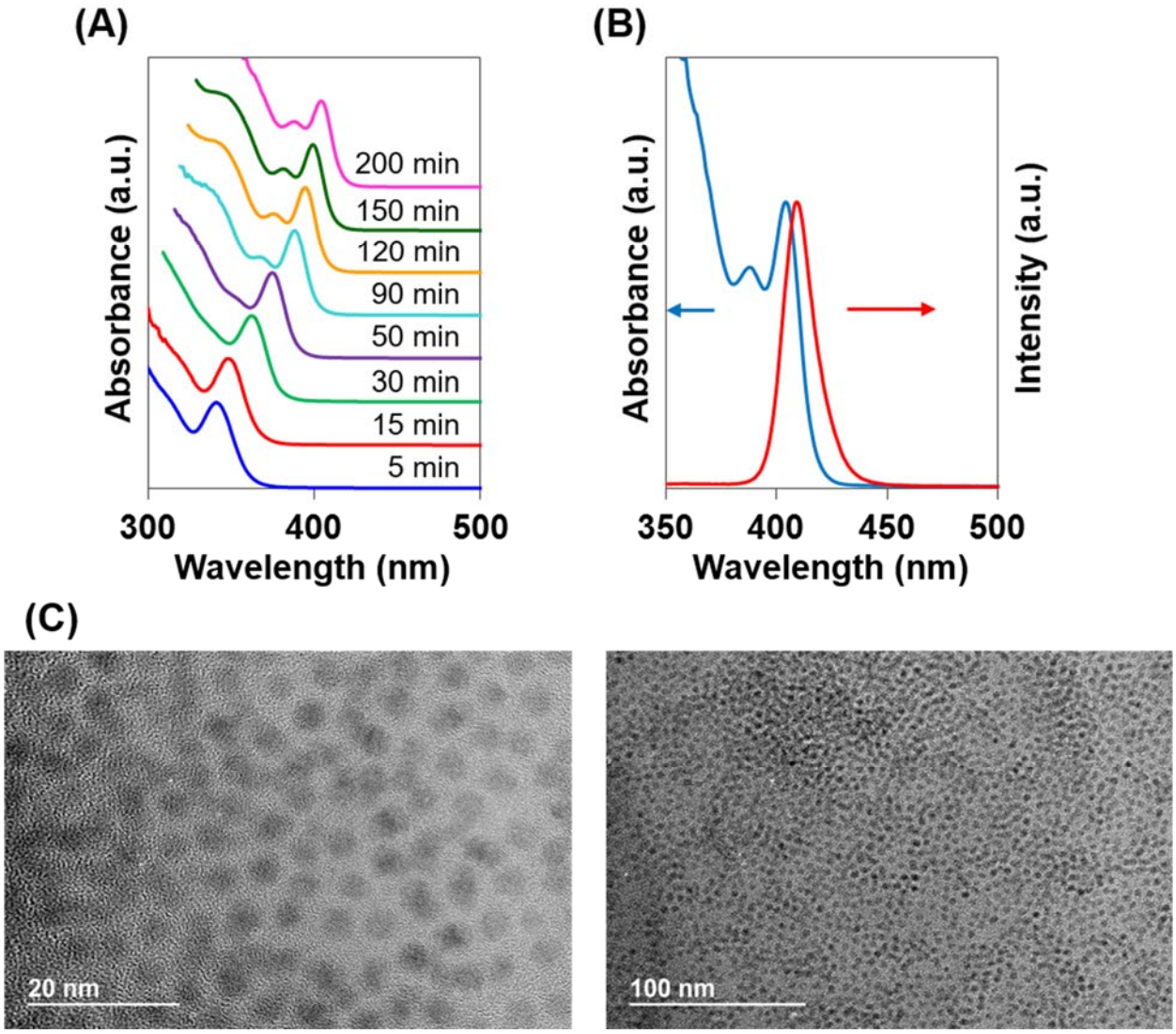

Figure S1. (A) Absorption spectra measured in chloroform during ZnSe core growth. (B) Absorption (blue line) and fluorescence (red line) spectra of ZnSe core after core growth. (C) High-resolution TEM images of the as-prepared ZnSe QDs (average size: 4.5 ± 0.41 nm).

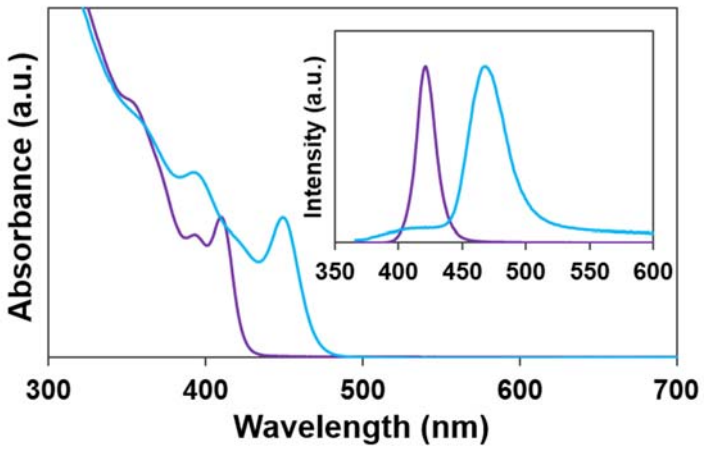

Figure S2. Absorption spectra of ZnSe QDs (purple line) and the product after cation exchange with Cd oleate (blue line) measured in chloroform. Inset shows the fluorescence spectra of ZnSe QDs (purple line) and the product after cation exchange with Cd oleate (blue line) measured in chloroform.

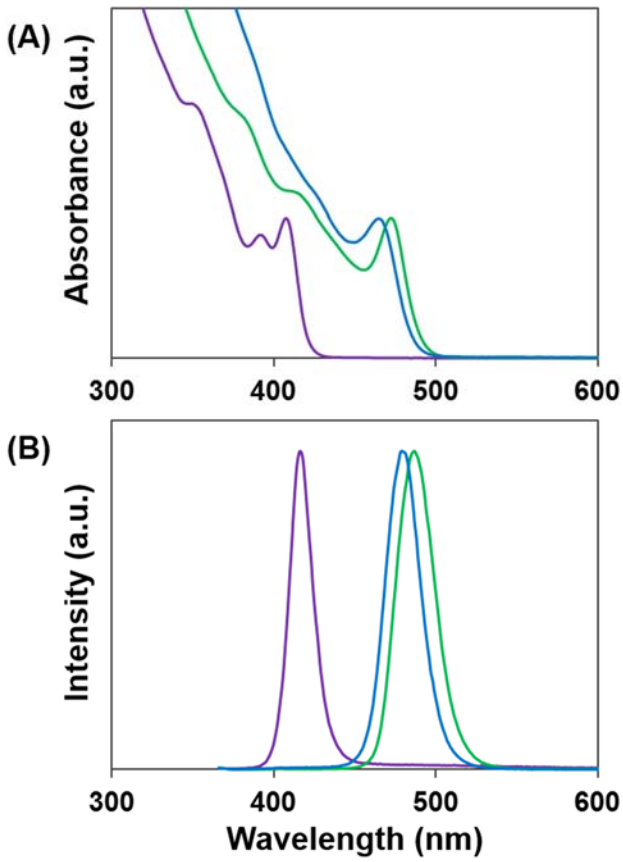

Figure S3. Absorption (A) and fluorescence (B) spectra of ZnSe core QDs (purple line), the QDs after overcoating with $Cd_{0.35}Zn_{0.65}S$ layers (green line), and the QDs after overcoating with ZnS layers (blue line). The QD samples are dissolved in chloroform.

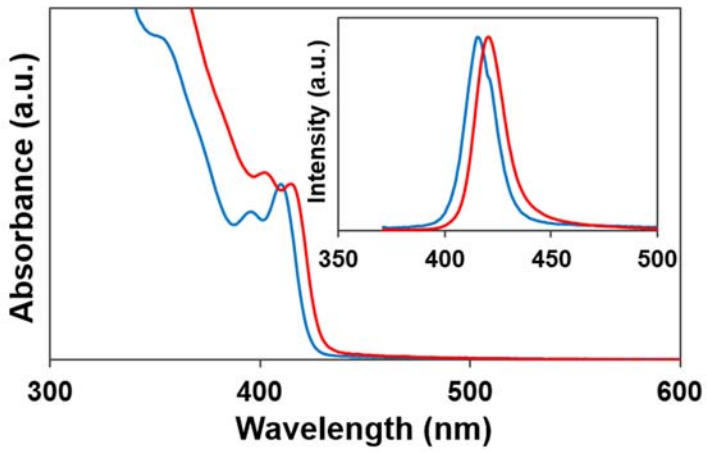

Figure S4. Absorption spectra of ZnSe core (blue line) and ZnSe/ZnS (red line) QDs measured in chloroform. Inset shows the fluorescence spectra of ZnSe core (blue line) and ZnSe/ZnS (red line) QDs.

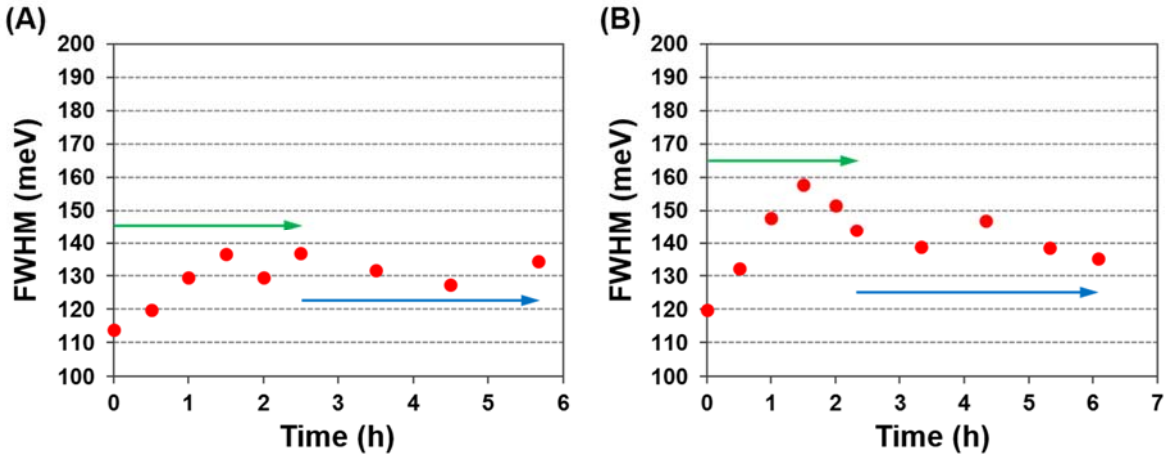

Figure S5. Examples of FWHM evolutions monitored during the direct overcoating of ZnSe cores with $Cd_yZn_{1-y}S$ and ZnS layers: (A) ZnSe/$Cd_{0.4}Zn_{0.6}$S(4ML)/ZnS(2ML); (B) ZnSe/$Cd_{0.35}Zn_{0.65}$S(4ML)/ZnS(3ML). The green and blue arrows indicate the process of $Cd_yZn_{1-y}S$ overcoating and ZnS overcoating, respectively.

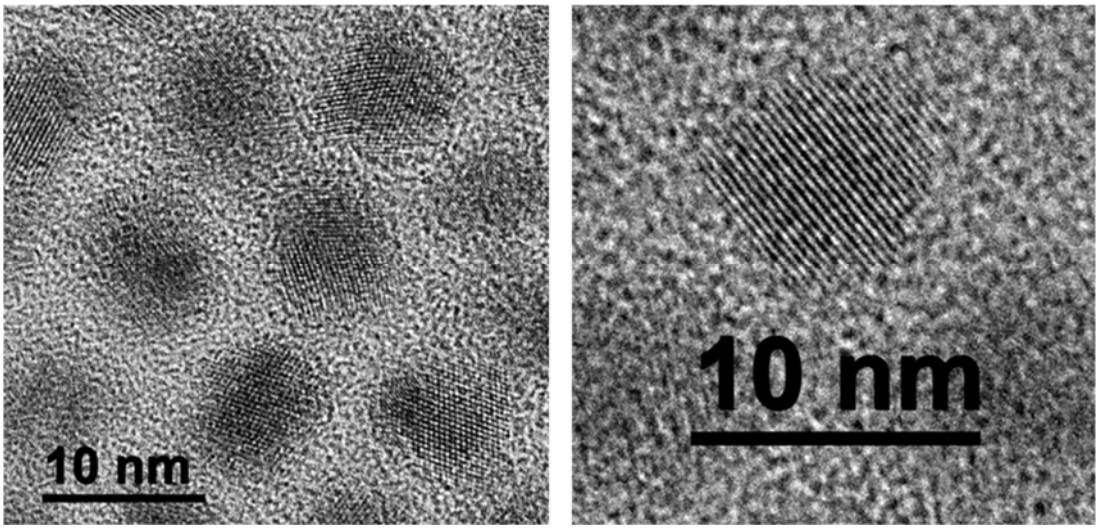

Figure S6. High-resolution TEM images of the as-prepared ZnSe/$Cd_{0.25}Zn_{0.75}S$/$Cd_{0.1}Zn_{0.9}S$/ZnS QDs (average size: 8.6 ± 0.7 nm).

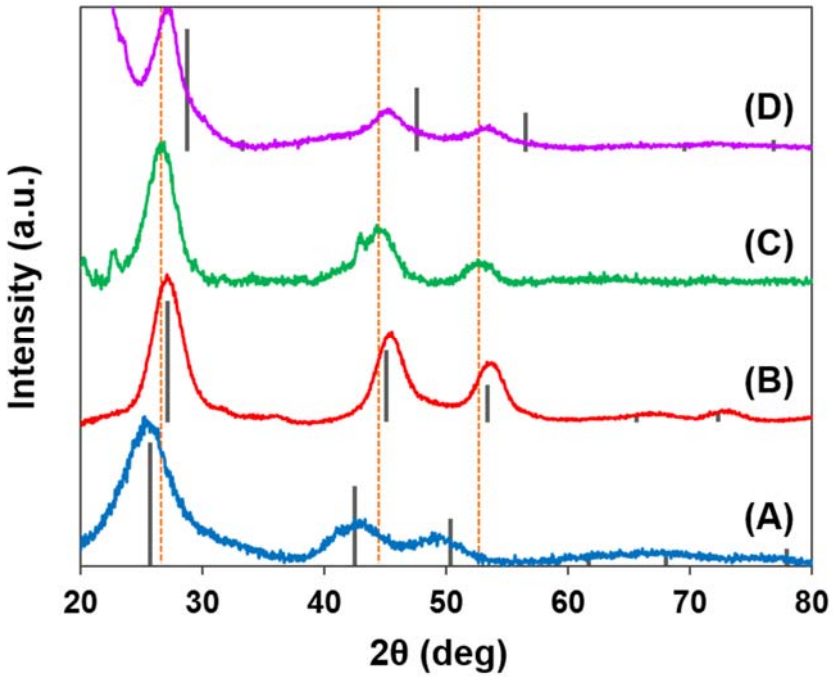

Figure S7. Powder X-ray diffraction patterns of (A) zinc blende CdSe cores, (B) ZnSe cores, (C) $Cd_xZn_{1-x}Se$ cores, and (D) $Cd_xZn_{1-x}Se/Cd_{0.2}Zn_{0.8}S$/ZnS core/shell QDs. The markers on each pattern indicate the bulk diffraction peak positions and the relative intensities for (A) zinc blende CdSe, (B) zinc blende ZnSe and (D) zinc blende ZnS, respectively. The vertical dotted lines show the main diffraction peak positions of the $Cd_xZn_{1-x}Se$ cores.

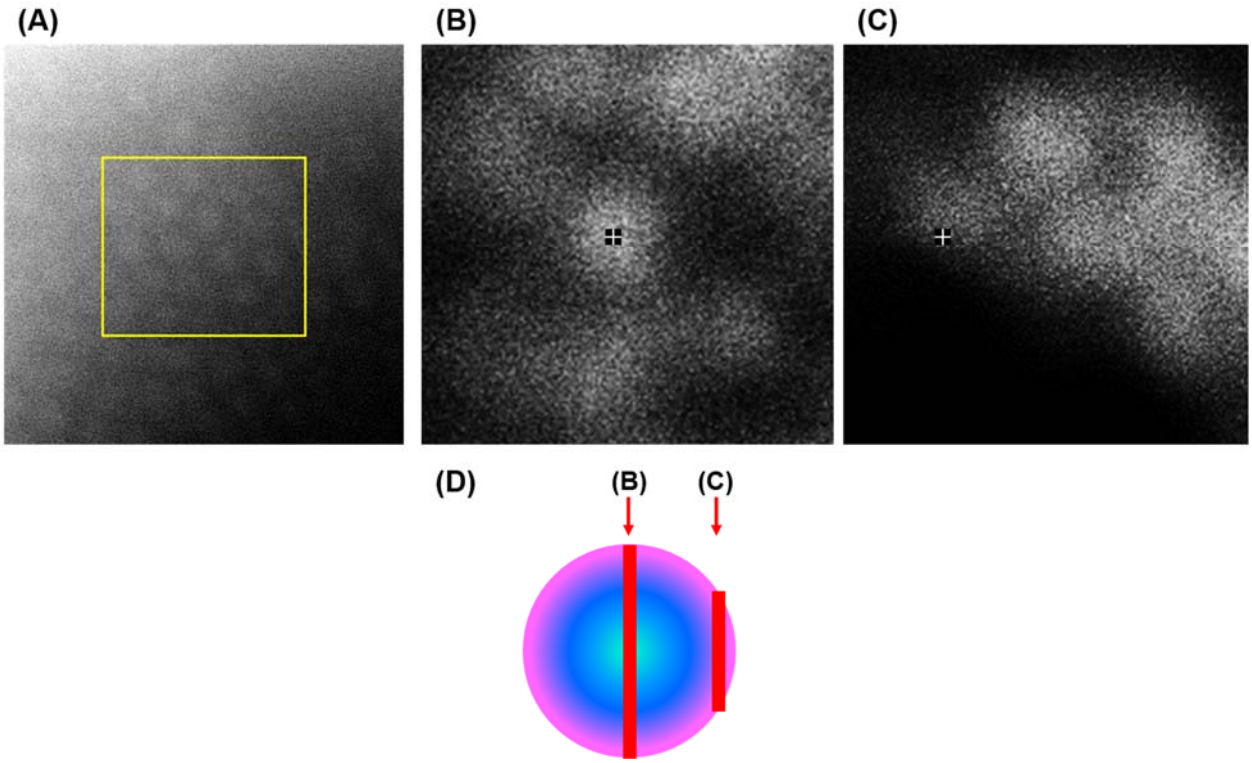

Figure S8. Representative STEM images of ZnSe/$Cd_{0.4}Zn_{0.6}S$/ZnS QDs. The EDX spectra were measured for either large area including multiple QDs (A) or single QDs (B and C). For the EDX spectra of single QDs, the electron beam (~0.5 nm size) was focused on either center (B) or edge (C) of single QD, and the spectra were measured at the marked positions (cross hairs). (D) Schematic representation of the probing regions of center (B) and edge (C) of single core/shell QD for the EDX analysis. The red rectangular areas at the center and edge are probed by incident beams (red arrows).

Table S1. Calculated and experimental atomic percentages of the chemical compositions of ZnSe/$Cd_{0.4}Zn_{0.6}S$/ZnS QDs measured by STEM-EDX.

Elements	Calculated	Experimental					
	Whole area	Whole area		Center		Edge	
	(%)	Average (%)	S.D.[a] (%)	Average (%)	S.D.[a] (%)	Average (%)	S.D.[a] (%)
S	43.1	35.2	2.5	32.0	1.9	39.2	3.2
Zn	44.3	51.1	3.9	46.1	2.6	50.9	4.9
Se	6.9	6.2	0.4	11.0	1.2	1.5	0.9
Cd	5.7	7.5	0.4	10.9	1.9	8.4	2.1

[a] Standard deviation.

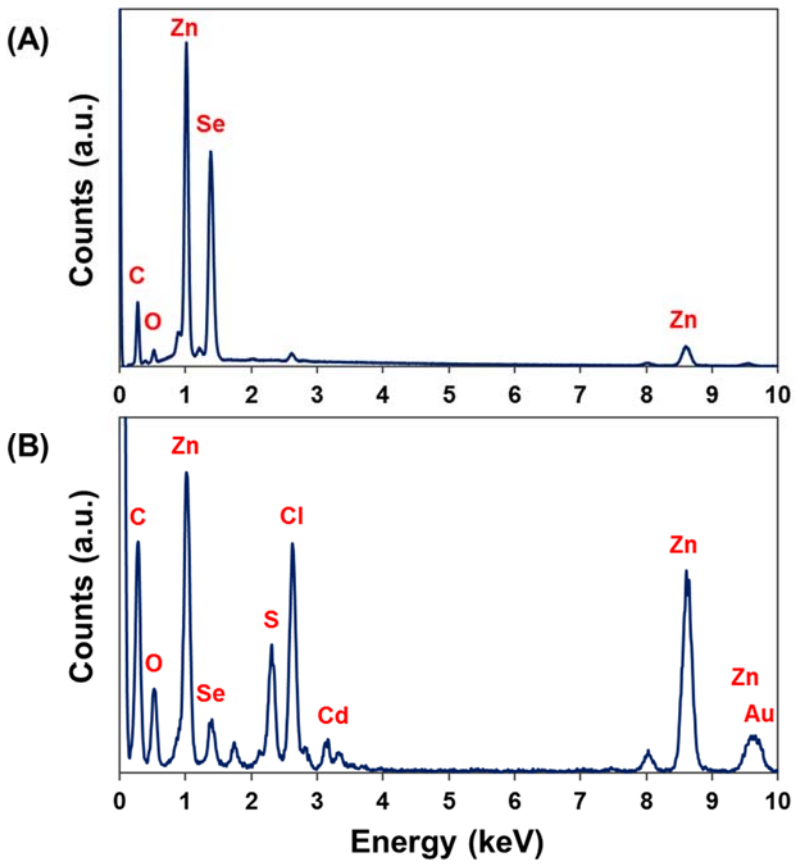

Figure S9. EDX spectra of (A) ZnSe core and (B) ZnSe/$Cd_{0.4}Zn_{0.6}S$/ZnS QDs.

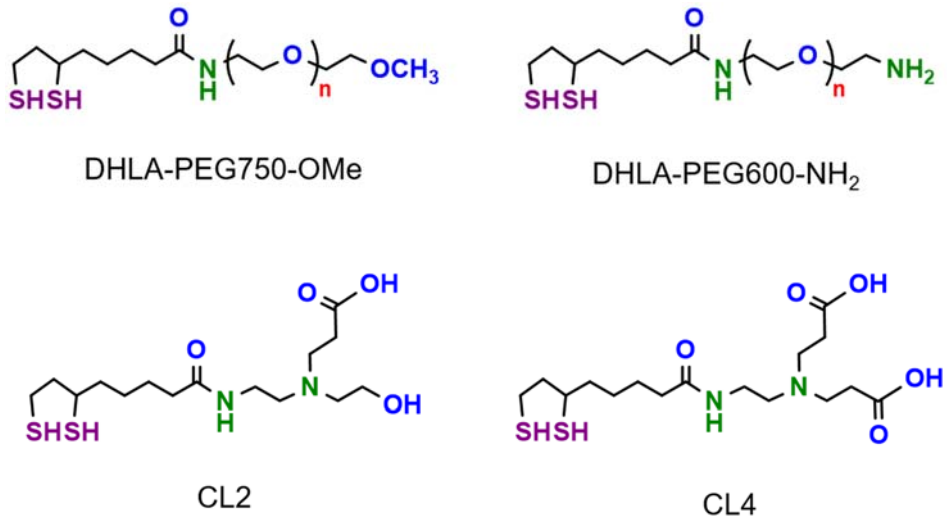

Figure S10. Chemical structures of the hydrophilic surface ligands used in this study.

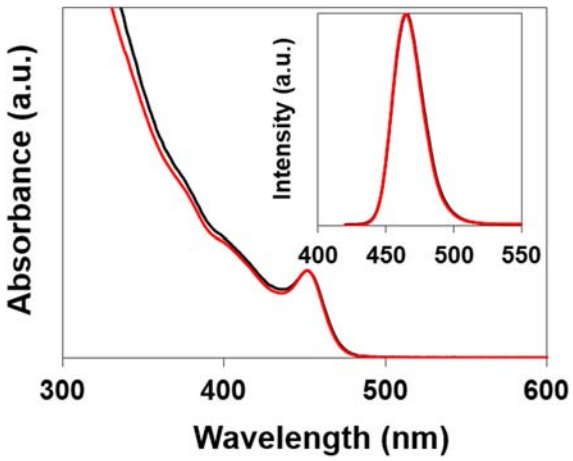

Figure S11. Absorption spectra of ZnSe/$Cd_{0.3}Zn_{0.7}S$/ZnS QDs with the original hydrophobic ligands in toluene (black line) and with the compact hydrophilic ligand CL2 in H_2O (red line). Inset shows the corresponding fluorescence spectra.

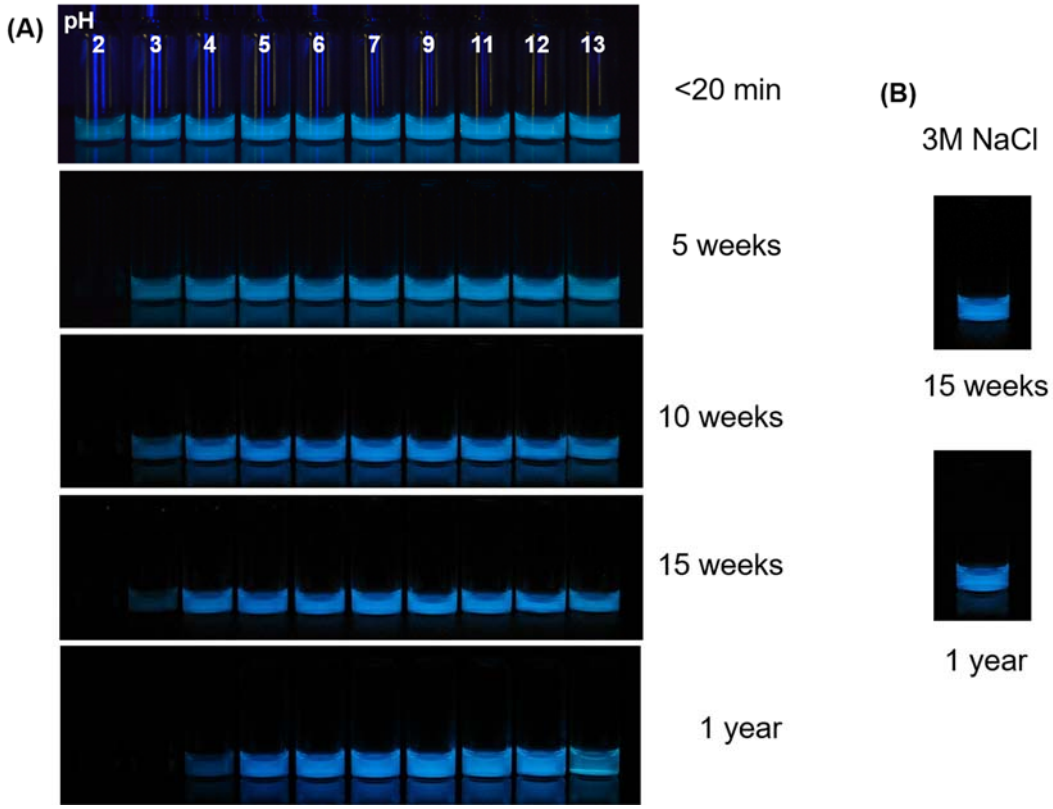

Figure S12. (A) Fluorescence images for a set of 0.5 μM QDs coated with DHLA-PEG750-OMe in different buffers at pH 2~13. 483 nm emitting CdZnSe/$Cd_{0.1}Zn_{0.9}S$/ZnS core/shell QDs were used and excited with a UV lamp at 365 nm. Images were taken at the indicated period of time after sample preparation. (B) Fluorescence images for 0.5 μM QDs coated with DHLA-PEG750-OMe in 3 M NaCl solution. Images were taken at the indicated period of time after sample preparation.

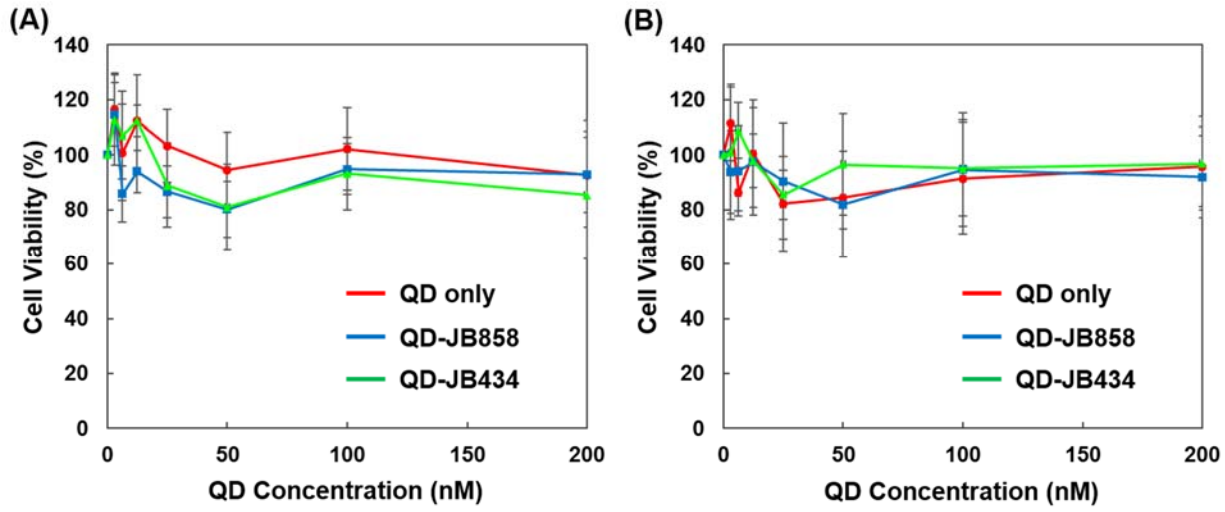

Figure S13. Cytotoxicity assay results demonstrating the effects of 483 nm emitting CdZnSe/$Cd_{0.1}Zn_{0.9}S$/ZnS core/shell QDs (red line), the QD-JB858 conjugates (blue line) and the QD-JB434 conjugates (green line) on cellular proliferation of COS-1 cells: (A) the QDs coated with DHLA-PEG750-OMe; (B) the QDs coated with CL4. COS-1 cells were incubated with the materials for 30 min, washed, and subsequently cultured for 48 h prior to the viability assay. Each data point represents the mean ± standard deviation of multiple measurements.

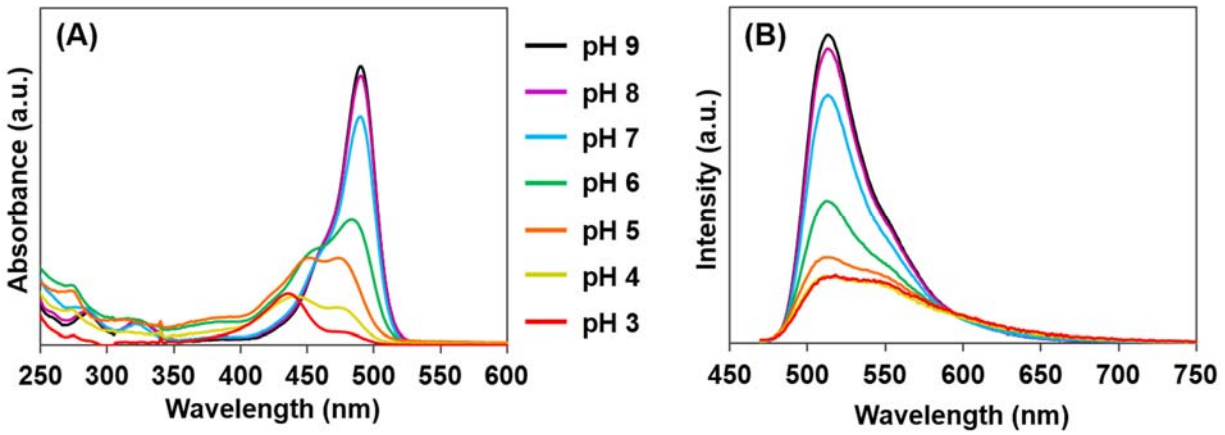

Figure S14. pH-dependent absorption (A) and fluorescence (B) spectra of fluorescein.

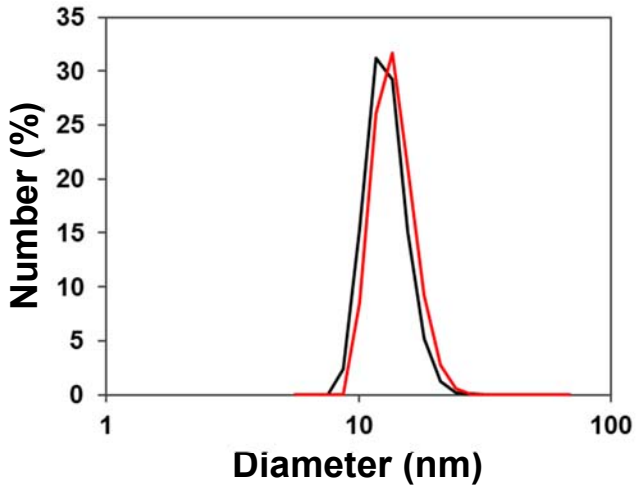

Figure S15. Hydrodynamic size distribution of 464 nm emitting $ZnSe/Cd_{0.3}Zn_{0.7}S/ZnS$ core/shell QDs coated with DHLA-PEG750-OMe:DHLA-PEG600-NH_2 (4:1) (black line, 13.0 ± 0.5 nm) and the QD-FITC conjugates (red line, 13.9 ± 0.2 nm) in water (pH 9).

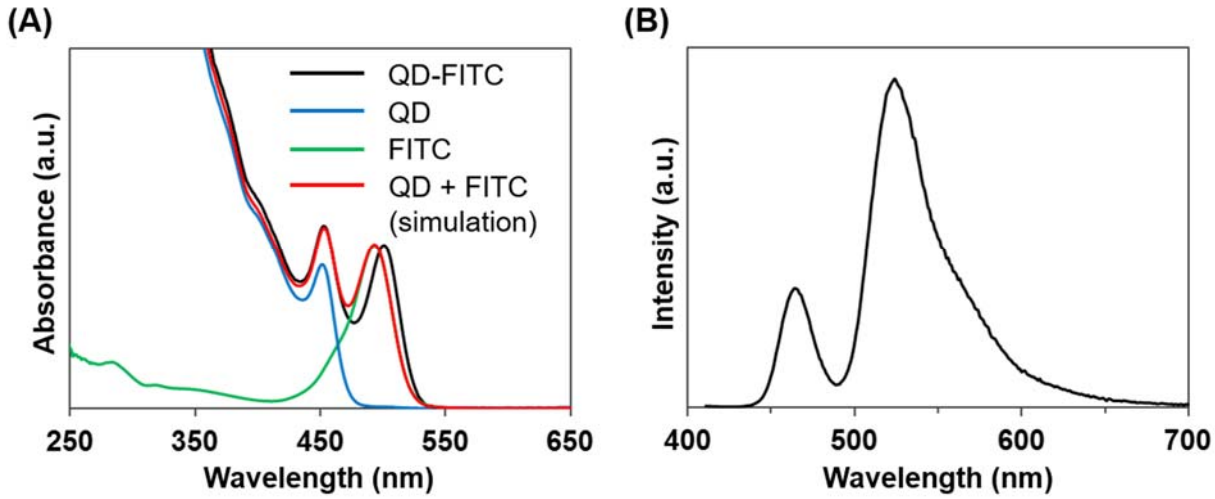

Figure S16. (A) Absorption spectra of the QD-FITC conjugates (black line), the 464 nm emitting $ZnSe/Cd_{0.3}Zn_{0.7}S/ZnS$ QD (blue line) and FITC (green line) in pH 9 buffer along with the simulated spectra (red line) of the QD-FITC conjugates via simple summation of the individual QD and FITC spectra. (B) Fluorescence spectra of the QD-FITC conjugates in pH 9 buffer excited at 400 nm.

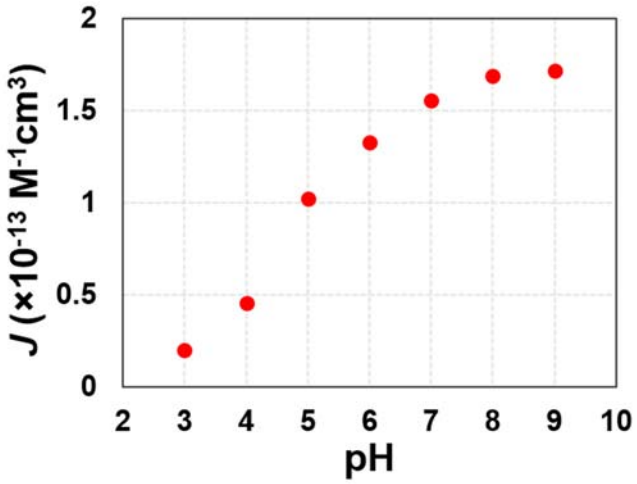

Figure S17. Plot of pH-dependent spectral overlap integral between the fluorescence spectra of 464 nm emitting ZnSe/$Cd_{0.3}Zn_{0.7}S$/ZnS QDs and the absorption spectra of fluorescein.

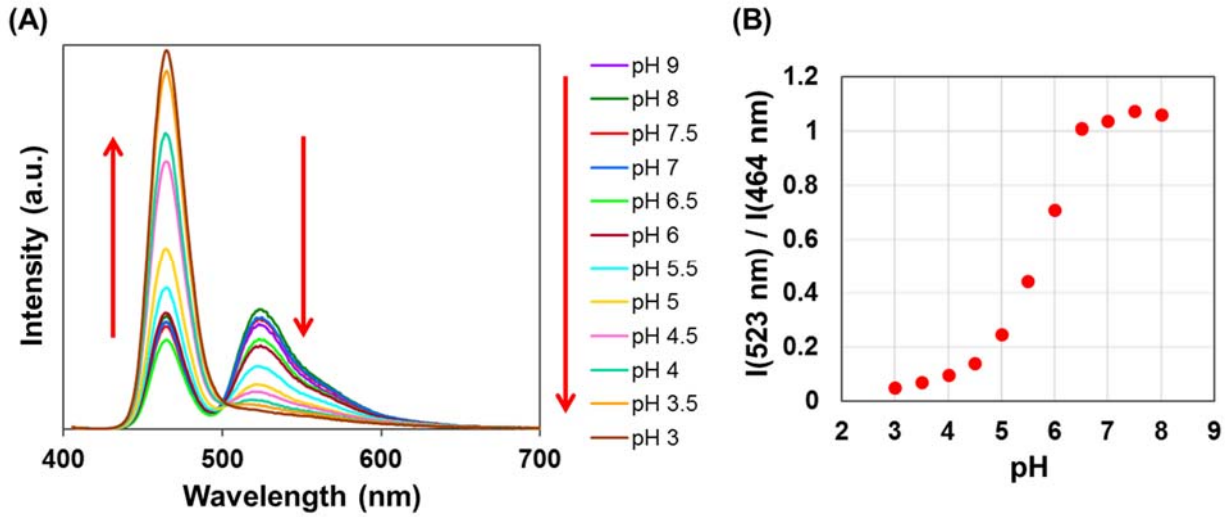

Figure S18. (A) Fluorescence spectral change of the QD-FITC conjugates in different pH buffers. 464 nm emitting QDs coated with DHLA-PEG750-OMe:DHLA-PEG600-NH_2 (4:1) were used, and the FITC/QD ratio in this system was estimated to be 8.0. The sample was excited at 400 nm. (B) Plot of the FRET ratio (I(523 nm)/I(464 nm)) of the QD-FITC conjugates as a function of pH.

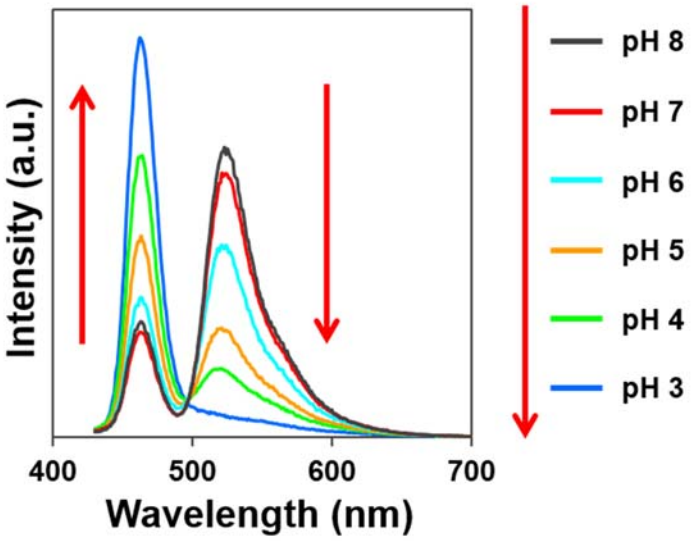

Figure S19. Fluorescence spectral change of the QD-FITC conjugates self-assembled with JB858 in DPBS buffers with different pHs. 464 nm emitting QDs coated with DHLA-PEG750-OMe:DHLA-PEG600-NH_2 (4:1) were used, and the FITC/QD ratio in this system was estimated to be 15.3. The sample was excited at 400 nm.