Supporting Information

Thiolactone Ring Dynamics in Dimeric Lipids Enable pH-Switchable Supramolecular Tuning in Surface-Engineered Quantum Dots

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Experimental Methods.

Chemicals.

Copper acetate [$Cu(OAc)_2$, 99.99%], indium acetate [$In(OAc)_3$, 99.99%], zinc acetate [$Zn(OAc)_2$, 99.99%], 1,2-octadecanediol, 1-dodecanethiol (DDT, 98%), 1-octadecene (ODE, 90%), Selenium dioxide (99.8%), tri-n-octylphosphine (TOP, technical grade, >85:0%), oleic acid (OA, 90%), oleylamine (OAm, 97%), zinc diethyldithiocarbamate [$Zn(S_2CNEt_2)_2$, 97%] and n-decane (98%) were purchased from the best known commercial resources and were used without further purification. The water used in all experiments had a resistivity higher than 18.2 MΩ·cm. The sulfur stock solution was prepared by dissolving 0.3 mmol (0.009 g) S in 0.5 mL OAm and 1.5 mL ODE under inert atmospheric conditions. To obtain a clear solution, the mixture was heated either in a vial or flask to 100-120 °C.

Synthesis of CdSe/ZnS Core-Shell Quantum Dots.

CdSe/ZnS core-shell quantum dots were synthesized according to a modified report.[1, 2] Cadmium oleate, $Cd(C_{17}H_{33}COO)_2$, was prepared using a literature procedure.[1] Typically, a 50 mL three-necked round-bottomed flask was loaded with 168.8 mg (0.25 mmol) of cadmium oleate, 27.7 mg (0.25 mmol) of SeO_2, 71.6 mg (0.25 mmol) of 1,2-octadecanediol, and 15 mL of ODE. The reaction mixture was quickly (within 8 min) heated to and maintained at 220 °C under N_2 atmosphere, and the CdSe core was allowed to grow for 5 min. The reaction was then quenched by adding 25 mL of 1-butanol, and the quantum dots (QDs) were precipitated by adding 20 mL of acetone and 20 mL of methanol. Sequentially, the QDs were isolated by centrifugation and washed with 20 mL of 1-butanol and 20 mL of methanol. Then a 50 mL three-necked round-bottomed flask was loaded with a solution of 0.5 μmol of CdSe in 10 mL of ODE, 3 mL of OAm, and 3 mL of TOP. To this 2.5 μmol of crystalline $Zn(S_2CNEt_2)_2$ was added, which was needed to grow two monolayers of ZnS. Under a nitrogen atmosphere, the reaction mixture was slowly (within approximately 75 min) heated at 110-120 °C for about 1 h. The mixture was allowed to cool to room temperature, and the QDs were precipitated by adding 25

mL of 1-butanol, 20 mL of acetone, and 20 mL of methanol. The QDs were isolated by centrifugation and repeatedly washed with 20 mL of 1-butanol and 20 mL of methanol.

Synthesis of $CuInZnS_2$ (CIZS) Quantum Dots.

CIZS quantum dots were also prepared according to a modified literature method.[3, 4] Typically, 0.01 mmol (0.0012 g) $Cu(OAc)_2$ and 0.1 mmol (0.029 g) $In(OAc)_3$ were taken in a three-necked flask along with 2 mL DDT, 0.6 mmol OAm and 5 mL ODE. The reaction mixture was then degassed by purging Argon for 15 minutes. The temperature of the mixture was then raised to ~130 °C till a clear solution was obtained. Then 2 mL of sulfur stock solution was injected into the flask. The resulting solution was annealed for five more minutes. Meanwhile, the color of the solution darkened, indicating the formation and growth of the nanoparticles. Further 0.1 mmol (0.018 g) $Zn(OAc)_2$ dissolved in 0.5 mL OAm and 1.5 mL ODE was injected into the reaction mixture, and the temperature was increased to 200 °C. The reaction was annealed for 30-40 minutes to allow effective diffusion of Zn into the CIS nanocrystals. The reaction mixture was then cooled down to room temperature, and the nanocrystals were purified using ethanol (10 mL) and acetone (10 mL).

Synthesis of the Dimeric Version of Palmitoyl HomoCysteine (diPHC) Lipid.

The dimeric version of palmitoyl homocysteine (diPHC) lipid was synthesized according to a modified literature protocol.[5] Typically, the synthesis started with the benzyl protection of the carboxylic acid groups of D-tartaric acid, followed by the palmitoylation of the free hydroxyl groups. The synthesis continued with the debenzylation and consecutive esterification of the carboxylic acid groups with N-hydroxy succinimide. Then the portions from the two stock solutions of N-hydroxy succinimide ester (1 gm, 1.2 mmol) in 20 mL of THF and homocysteine hydrochloride (584 mg, 3.8 mmol) in 20 mL of distilled water were added alternatingly into a stirred mixture of 40 mL of 0.2 M Na_2CO_3-$NaHCO_3$ buffer, pH 9.0 and 40 mL of THF. The mixture was stirred at ambient temperature for 16 h and then acidified with 1 M HCl to pH 2-3. Then the mixture was warmed to 60 °C and bubbled with N_2 to facilitate the precipitation of the thiolactone form of the compound. The product was collected and washed extensively with water. Recrystallization from methanol yielded the pure compound, diPHC, in 75% yield. FT-

IR (neat) (cm-1): 2920, 2848, 1652, 1530, 1230, 1208, 1154, 1128, 965, 719, 562. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 0.89 (t, J = 6.0 Hz, 6H), 1.25 (m, 48H), 1.65 (m, 4H), 1.98 (m, 4H), 2.38 (t, J = 7.2 Hz, 4H), 2.56 (t, J = 7.2 Hz, 4H), 4.55 (m, 1H), 4.68 (m, 1H), 6.55-6.62 (m, 2H). ^{13}C-NMR ($CDCl_3$, 400 MHz): δ: 14.08, 22.65, 25.56, 29.32, 29.36, 29.65, 31.86, 57.22, 80.08, 173.1, 174.5. ESI-MS: m/z calcd for (M + Na^+): 883.5152, found 883.5156.

Surface Modification of Quantum Dots with PHC lipids.

The CdSe/ZnS and CIZS core-shell quantum dots were initially solubilized in anhydrous chloroform. 6.5 mg of PHC was solubilized in 1 mL of 2:1 tetrahydrofuran:chloroform media and this solution of PHC was separately added to 200 ng/mL of CdSe/ZnS or CIZS solution. Then the mixtures were stirred for 36 hours under a nitrogen atmosphere at 45 °C. The mixtures were then centrifuged at 4000 g for 3 minutes, and supernatants were collected in each case. The supernatants were further centrifuged at 15000 g for 15 minutes. The supernatants still contained unbound PHC and substituted ligands (like TOP/TOPO, oleylamine for CdSe/ZnS, and 1-dodecanethiol for CIZS). These supernatants were then discarded, leaving the residual surface-modified quantum dots with a minimal amount of solvent at the bottom. The washing was repeated three consecutive times with the addition of THF followed by centrifugation to remove the 'loosely' bound PHCs from the surface-modified quantum dots. Alternatively, a catalytic amount of tetramethylammonium hydroxide could also be added to the supernatants before centrifuging them for 15000 g for immediate precipitation of PHC coated quantum dots (CdSe/ZnS_PHC and CIZS_PHC). For the aqueous suspension of the surface-modified QDs, the purified QDs were taken in an aqueous medium, vortexed, and sonicated for 5 minutes at room temperature.

Surface Modification of Quantum Dots with diPHC lipids.

The CdSe/ZnS and CIZS core-shell quantum dots were initially solubilized in anhydrous chloroform. 7.3 mg of diPHC was solubilized in 1 mL of tetrahydrofuran (THF), and this solution of diPHC was separately added to 200 ng/mL of CdSe/ZnS or CIZS solution. The mixtures were stirred for 36 hours under nitrogen atmosphere at 45 °C. The mixtures

were then centrifuged at 4000 g for 3 minutes, and supernatants were collected in each case. The supernatants were further centrifuged at 15000 g for 15 minutes. The supernatants contained unbound diPHC and substituted ligands (like TOP/TOPO, oleylamine for CdSe/ZnS, and 1-dodecanethiol for CIZS). These supernatants were then discarded, leaving the residual surface-modified QDs with minimal solvent at the bottom. The washing was repeated three consecutive times with the addition of THF followed by centrifugation to remove the 'loosely' bound diPHCs from the surface-modified quantum dots. Alternatively, a catalytic amount of tetramethylammonium hydroxide could also be added to the supernatants before centrifuging them for 15000 g for immediate precipitation of diPHC coated quantum dots (CdSe/ZnS_diPHC and CIZS_diPHC). For the aqueous suspension of the surface-modified QDs, the purified QDs were taken in an aqueous medium, vortexed, and sonicated for 5 minutes at room temperature.

Dispersions of quantum dots.

The ligand exchange reaction was performed in organic solvent. The lipids PHC and diPHC are soluble in chloroform. For making dispersion of lipidated QDs in chloroform, QDs were ligand exchanged, centrifuged (15000g for 15 min), precipitated down and dispersed the pellet in chloroform by vortex and mild sonication. For dispersing the lipidated QDs in aqueous solution, the centrifuged pellet was dispersed in aqueous media. Similar process was followed to disperse the native QDs in aqueous suspension. We centrifuged the chloroform solution of as synthesized QDs at 15000g for 15 min. The supernatant was discarded, leaving the residual as synthesized QDs with minimal solvent. We purged argon gas to evaporate the remaining chloroform followed by vacuum drying of the pellet to remove any traces of organic solvent. Water was added immediately and vortexed followed by sonication to make a dispersion of the as synthesized QDs. It is to be noted that the un-lipidated QDs are not stable in aqueous dispersion, however, the stability of the lipidated QDs suspensions are similar in organic and more stable in aqueous media.

Characterization Methods.

^{1}H NMR Spectroscopy.

^{1}H-NMR spectra were recorded in FT-NMR Bruker DPX 400 MHz NMR spectrometer. All the chemical shifts were reported in ppm (parts per million).

Transmission Electron Microscopy (TEM).

Morphology and particle sizes of the synthesized quantum dots were determined using transmission electron microscopy (TEM) with a JEOL ARM200f TEM equipped with a Field Emission Gun operating at 200 kV. For FEG-TEM, the samples were prepared by drop-drying diluted chloroform or aqueous suspension of the samples onto a 300 mesh carbon-coated copper grids. For FEG-TEM, we drop-coated the media containing QDs (at pH 5 to 7) over the grid. The excess aqueous media on the grids was wicked away using tissue paper and we instantly attached the container having grids with a desiccator to a vacuum line to evaporate the water rapidly from the grids to avoid degradation of the TEM grids. The lipidated QD assembly patches were visualized in aqueous media using Cryo- transmission electron microscopy with a JEOL JEM-2100PLUS Cryo-TEM. An aqueous suspension of lipidated QDs in its corresponding pH buffer was taken to observe directly in liquid state using Cryp-TEM. About 3 uL of the suspension was loaded on a 300 mesh copper grid within a GATAN Cryo plunging system (GATAN Cp3). The grid was undergone a plotting time of 4 seconds and immediately plunged into liquid ethane maintained within a liquid nitrogen bath (temperature of liquid ethane was prescisely maintained at ~ -170 °C). The Cryo-quenched grid was then immediately transferred to high-tilt cryo sample holder (GATAN 914.5) maintained at a temperature of -190 °C within liquid N2 bath. Then the sample holder with the grid was immediately transferred into the cryo-tem coloumn (JEOL JEM 2100 plus) maintained at ~-178-180 °C. Finally, the grid was studied under 120 kV electron accleratаion voltage with minimum possible low electron dosing. Analysis of the TEM images was performed with Gatan Digital Micrograph software. The mean diameter and the size distribution of each sample were obtained by statistical analysis from the recorded images with ImageJ software.

UV-Visible Spectroscopy. Absorbance spectra were recorded in Shimadzu UV-2600 UV-Vis spectrophotometer and Biotek epoch 2 microplate reader using 200 μL quartz

cuvette and 96 well plates, respectively. Data were processed using UVprobe 2.52 for Shimadzu UV-Vis spectrophotometer and Gen5 for Biotek Epoch 2.

Fluorescence Spectroscopy.

Fluorescence spectra were recorded in Horiba Fluromax-4 spectrofluorometer equipped with micromax384. Samples were measured in a 200 μL microfluorometer cell for Fluoromax-4. Micromax384 was used only for recording samples in 96 well plates. Data were processed with FluorEssence v3.8 in both cases. The quantum dots were irradiated with 365 nm Hg lamp for different time points for the evaluation of the photostability of the nanoparticles.

Fluorescence Lifetime Measurements.

Fluorescence lifetime spectra were measured in Horiba Jobin Yvon IBH with JY-IBH 5000M monochromator. Time-resolved fluorescence measurements were done with an excitation wavelength of 375 nm by a source of NaboLED-375L (pd <200ps) for both the quantum dots. IRF of the time correlated single photon counting (TCSPC) was 45 ps. The emission wavelength was set at 584 nm and 605 nm for CdSe/ZnS and CIZS core-shell QDs. The pico-timing amplifier and discriminator was Ortec 9327. The sample solutions were taken in cuvette separately, and the detection was done by an MCP PMT Hamamatsu R3809 multichannel plate multiplier. For data acquisition and overall controlling Data Station v2.3 and for fluorescence decay analyses, DAS6 software was used. Time calibrations were calculated using multi-exponential fitting.

For data acquisition and overall controlling, Data Station v2.3 and for fluorescence decay analyses, DAS6 software was used. Time calibrations were calculated using multi-exponential fitting.

DAS6 uses the following parameters. B value = pre-exponential function which relates the presence of an emitting species and T value = lifetime. The magnitude of the B values returned in the fit are dependent on the re-convolution used in fitting the data. For our multi-exponential fitting, B values are normalized. If we consider the decay to be represented as a sum of exponential components

$$I(t) = \sum_{i}^{n} B_i \exp(-t / T_i)$$

Then the normalized pre-exponential value is

$$B_i = \frac{B_i}{\sum_{i=1}^{n} B_i}$$

T values (lifetimes) has been interpreted as an average lifetime. The average lifetime was calculated as the simple sum of normalised pre-exponential multiplied by the lifetime.

$$\tau_{ave} = \sum_{i=1}^{n} B_i T_i$$

This is the output of the average lifetime given by DAS6. For use in Stern-Volmer analysis, it was be obtained from

$$\tau_{ave} = \frac{\sum_{i=1}^{n} B_i T_i^2}{\sum_{i=1}^{n} B_i T_i}$$

The equations for the determination of average lifetime by multi-exponential fit was obtained from Horiba Jovin Yvon IBH DAS6 operation manual and has been added in the newly submitted version of the supporting information.

Quantum yield measurement.

The quantum yields have been determined with respect to rhodamine 6G, a reference standard of known QY. If same excitation wavelength, gain and slit bandwidth are applied for the samples, then the quantum yield can be calculated as

$$QY = QY_{ref} \frac{n^2 \ I A_{ref}}{n_{ref}^2 A I_{ref}}$$

Where, QY_{ref} = quantum yield of reference standard rhodamine 6G

n, n_{ref} = refractive indexes of the solvents (n = $CHCl_3$/H_2O, n_{ref} = Ethanol)

I = integrated fluorescence intensity of QDs

I_{ref} = integrated fluorescence intensity of rhodamine 6G

A = absorbance at excitation wavelength of QDs and

A_{ref} = absorbance at excitation wavelength of rhodamine 6G

Quantum yield of rhodamine 6G is 0.95 (emission range 560-580 nm, refractive index 1.45 in ethanol). Calculation was done using Fluortools a|e-UV-Vis-IR spectral analysis software running on MATLAB compiler runtime.

Fourier Transform Infrared Spectroscopy. FT-IR data were measured in Perkin Elmer Spectrum 100 instrument.

Dynamic Light Scattering (DLS) and Zeta Potential Measurements.

DLS and zeta potential measurements were performed at room temperature using a Malvern Zetasizer Nano ZS particle sizer (Malvern Instruments Inc., MA). Dust-free conditions were maintained when samples were prepared and examined. The mean hydrodynamic diameters were obtained from a Gaussian analysis of the intensity-weighted particle size distributions and reported.

Ellman's Assay.

The Ellman's assay was used to quantify free thiol groups. The reaction buffer consisted of 0.1 M sodium phosphate buffer (pH 8.0) containing 1 mM EDTA. Ellman's reagent (DTNB) was freshly prepared by dissolving 4 mg of DTNB in 1 mL of reaction buffer. A calibration curve was established using L-cysteine standards ranging from 0 to 200 nM. Absorbance values were recorded at 412 nm using a UV-Vis spectrophotometer, and the concentration of free thiols was determined using the established standard curve.

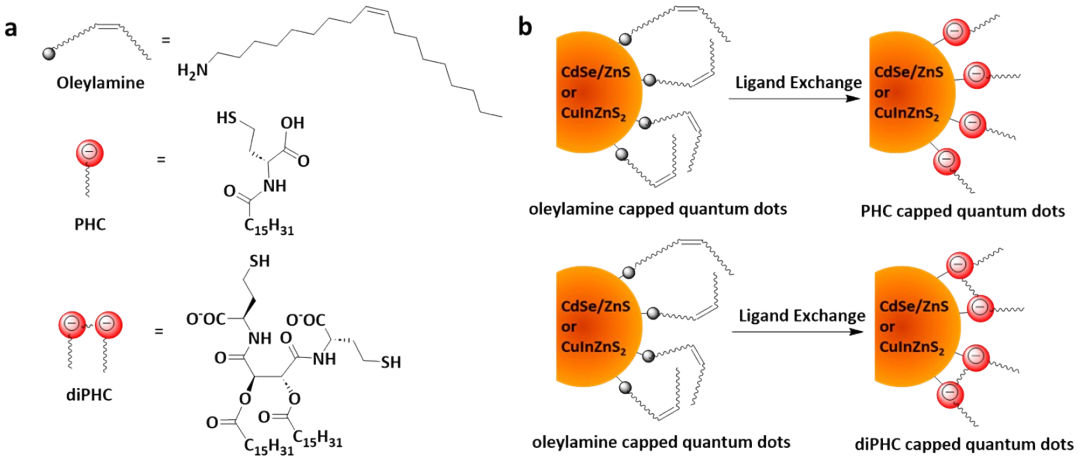

Figure S1. (a) Structural representation of n-oleylamine, PHC and diPHC; (b) Schematics for preparing PHC and diPHC capped CdSe/ZnS or $CuInS_2$/ZnS (CIZS) quantum dots from oleylamine capped quantum dots via ligand exchange protocol.

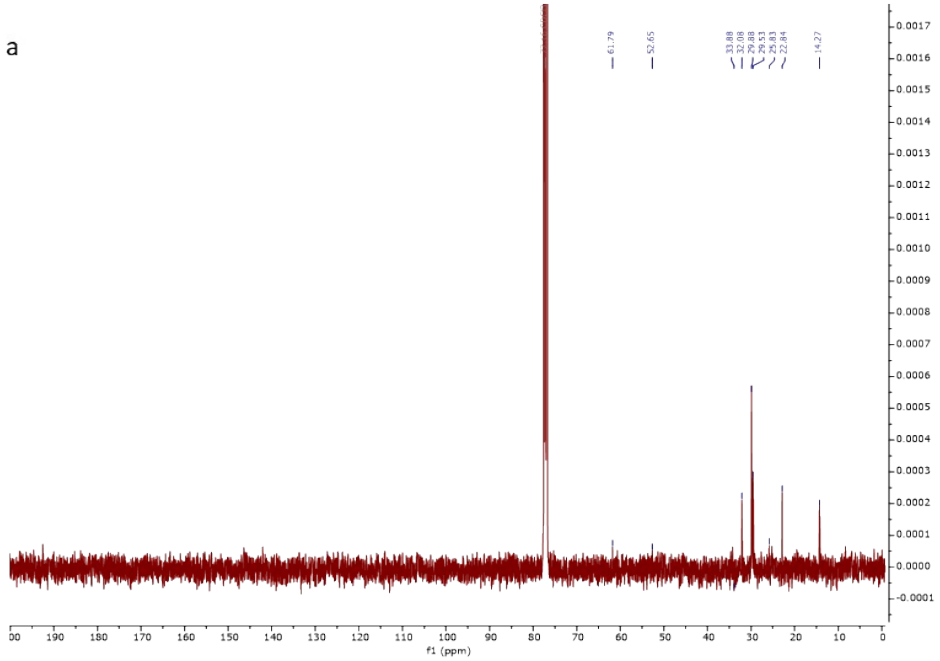

Figure S2. ^{13}C NMR of ligand-exchanged CIZS_diPHC

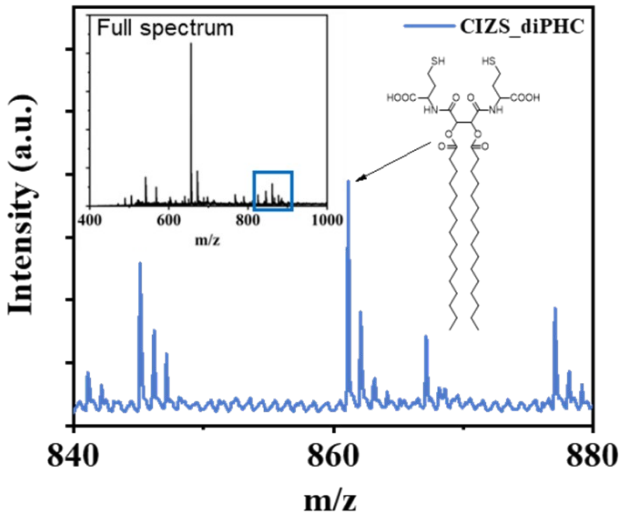

Figure S3 MALDI-TOF MS of ligand exchanged CIZS-diPHC exhibits the molecular ion fragments corresponding to the diPHC moiety (full spectrum in inset)

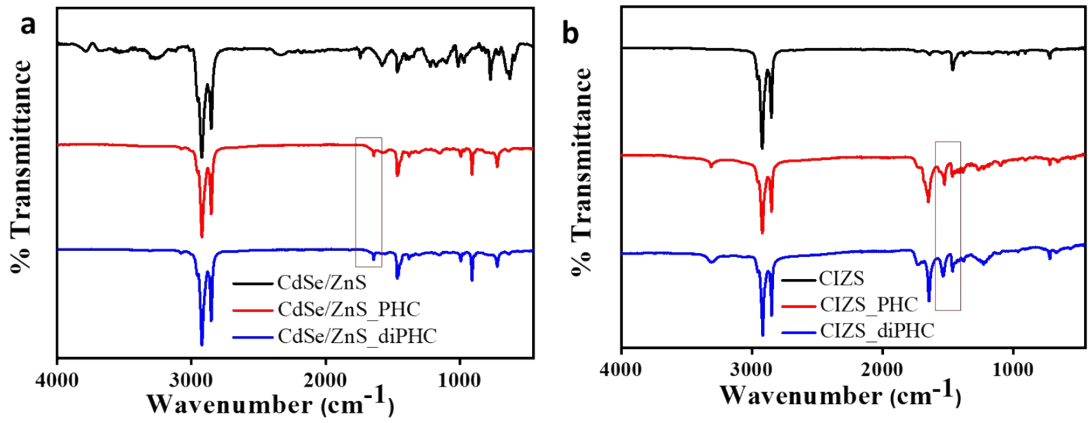

Figure S4 FT-IR spectra of PHC and GPHC capped (a) CdSe/ZnS and (b) CIZS quantum dots.

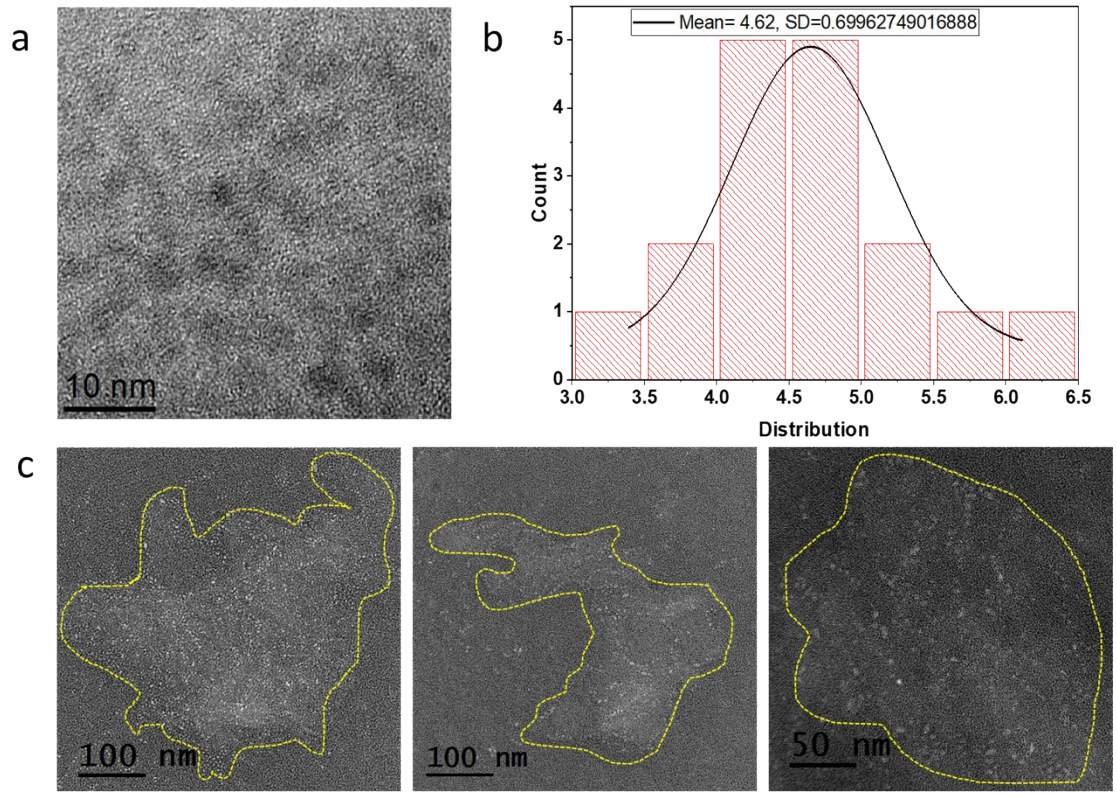

Figure S5. (a) TEM images with (b) size distribution (from TEM) of native CdSe/ZnS QDs

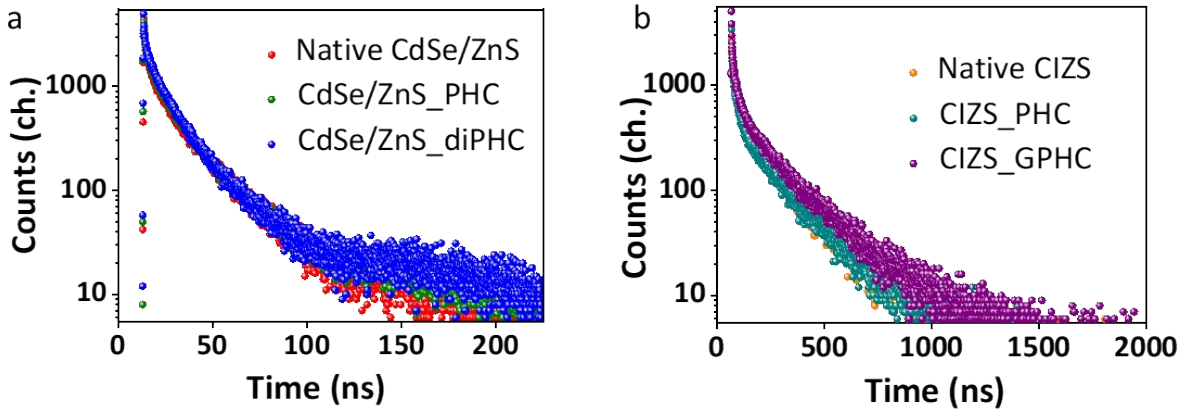

Figure S6. Fluorescence lifetime spectra of (a) CdSe/ZnS and (b) CIZS quantum dots are shown herein before and after capping with PHC and diPHC.

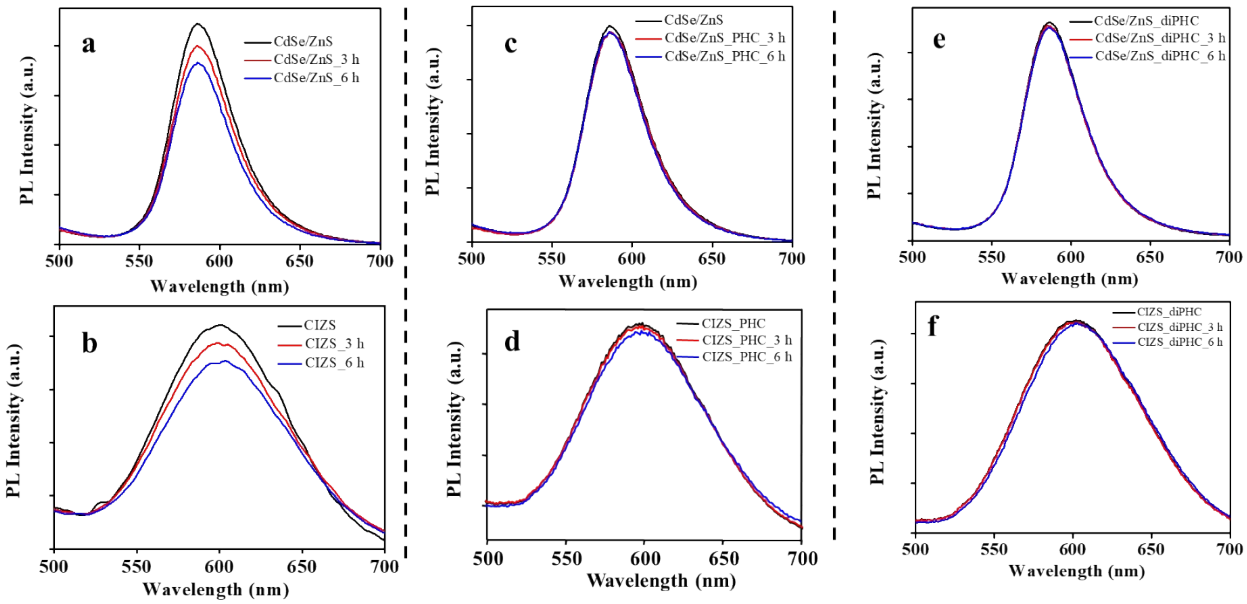

Figure S7. Demonstration of photostability of (a,c,e) CdSe/ZnS and (b,d,f) CIZS quantum dots before and after capping with PHC and diPHC. The photostability of the quantum dots are shown for a maximum of 6 hours in chloroform medium when irradiated with 365 nm, 6W Hg lamp.

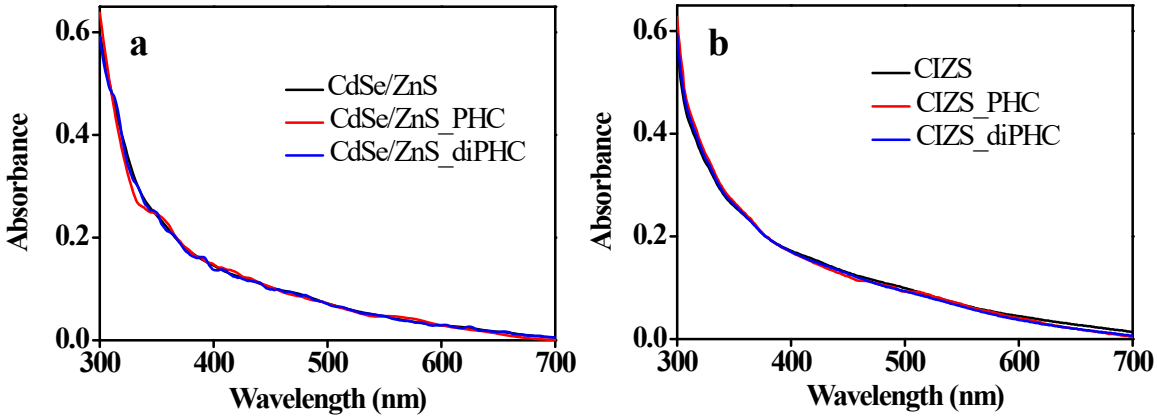

Figure S8. UV-Visible spectra of (a) CdSe/ZnS and (b) CIZS quantum dots in chloroform before and after capping with PHC and diPHC.

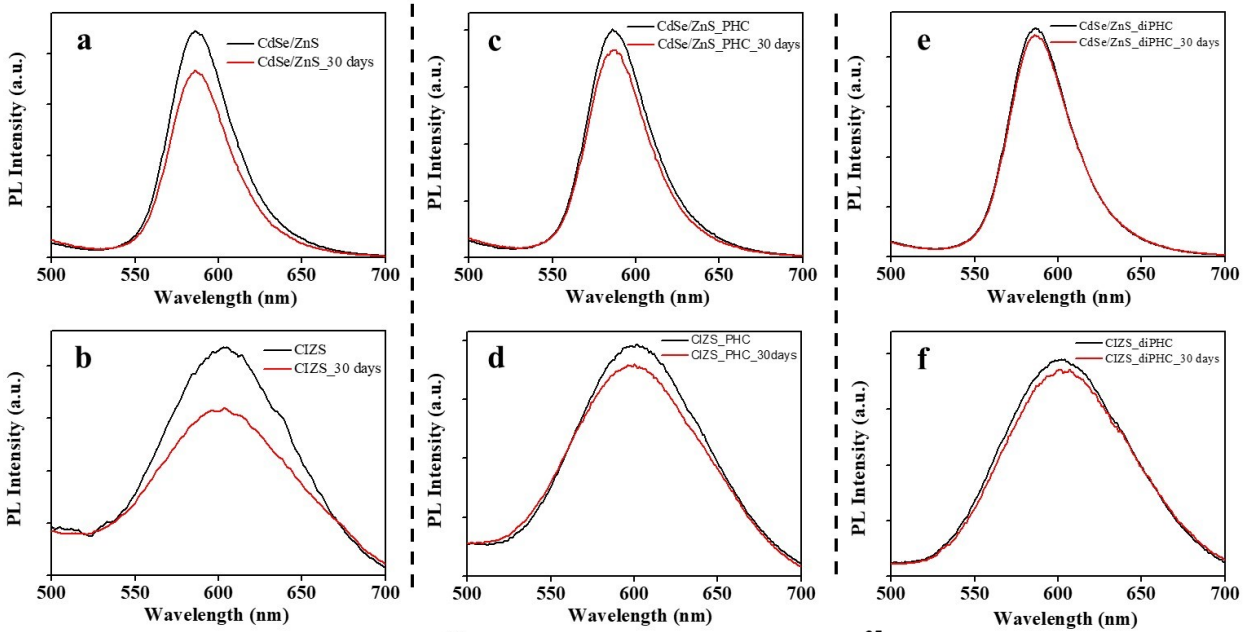

Figure S9. Demonstration of colloidal stability of (a,c) CdSe/ZnS and (b,d) CIZS quantum dots after capping with PHC and diPHC. The colloidal stability of the suspensions of quantum dots is shown up to 30 days in 1X PBS buffer (pH 7.4).

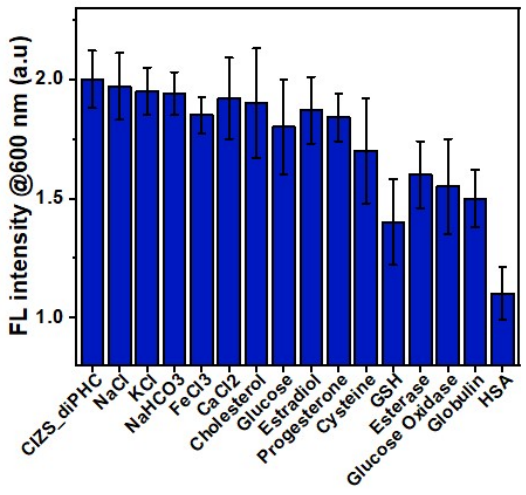

Figure S10. Change in fluorescence of CIZS_diPHC with different serum components.

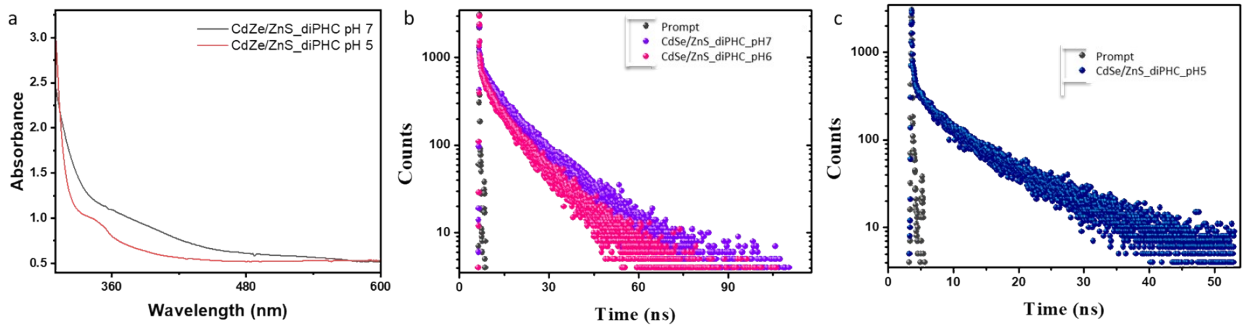

Figure S11. (a) UV-Vis absorption spectra of CdSe/ZnS_diPHC in neutral to acidic pH shows minimal change in absorbance spectra confirming inherent photophysical stability of the QDs. Time-resolved fluorescence of CdSe/ZnS in (b) pH 7, 6 and (c) pH 5 shows shortening in lifetime with differential multiexponential decay confirming intact core integrity.

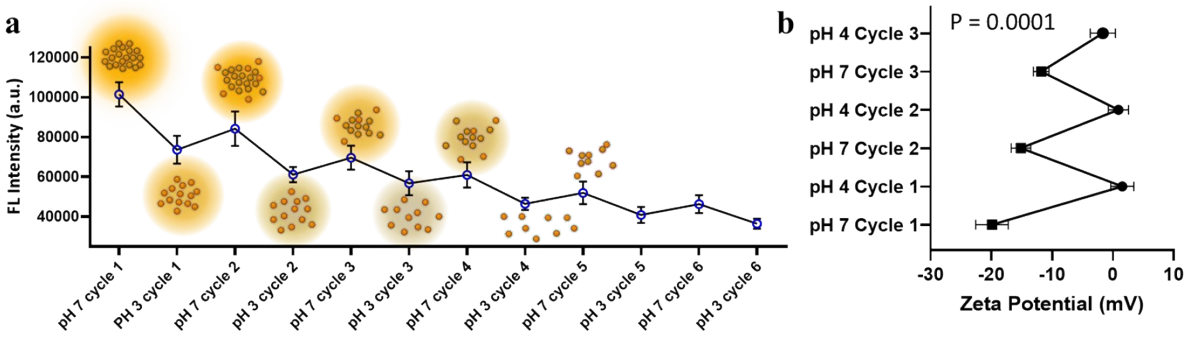

Figure S12. (a) Changes in fluorescence intensities with multiples pH decrease-increase cycle shows reversibility and gradual decrease in intensities with each cycle (b) Reversibility of zeta potential values of CIZS_diPHC with multiple pH decrease-increase cycles.

References

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