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Supporting Information

Generation of a library of non-toxic quantum dots for cellular imaging and siRNA delivery

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Keywords: Non-toxic Quantum Dots • Sonochemistry • siRNA delivery • Stem Cell imaging • Nanomaterial Library

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Materials: Silver Nitrate ($AgNO_3$, ≥ 99.0%), Indium (III) nitrate hydrate ($In(NO_3)_3.xH_2O$, 99.9%), Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$, Reagent grade), Sodium diethyldithiocarbamate trihydrate ($(C_2H_5)_2NCS_2Na.3H_2O$, Reagent grade) and Dodecylamine (98%) were purchased from Aldrich. 3-mercaptopropionic acid (≥ 99.0%) was purchased from Fluka. All other solvents were obtained from Fisher and used as received unless otherwise noted.

Physical Characterization of the ZAIS QDs: The morphology and size distribution of ZAIS QDs was determined using TEM analysis. The water-soluble ZAIS-QDs were drop cast on Holey-carbon grids (Electron Microscopy Sciences), allowed to dry overnight under vacuum and subsequently imaged using a JEOL JEM-2010F high-resolution transmission electron microscope operated at an accelerating voltage of 200 kV. Additionally, the polydispersity index (PDI) and hydrodynamic size of the ZAIS QDs was determined using DLS (Malvern Zetasizer Nano). Powder X-ray diffraction (XRD) analysis was carried out on a Bruker D8 X-ray diffractometer (GmbH, Karlsruhe, Germany) with CuKα radiation to determine the crystallinity of the ZAIS QDs. The powder samples were prepared by drying a chloroform solution of the ZAIS QDs *in vacuo*. The composition and elemental analysis of the ZAIS QDs was determined using an EDAX Eagle II Micro-EDXRF Spectrometer (EDAX Inc., Mahwah, NJ, USA). UV-Visible absorption spectra were recorded by a Varian Cary 5000 UVVis-NIR Spectrophotometer. Photoluminescence (PL) spectra were measured using a LS 55 Perkin–Elmer Luminescence Spectrometer.

Determination of PL stability of ZAIS QDs: A solution of the ZAIS QD (composition of x=0, y=0.2) in PBS (pH=7.4) was incubated at 37 °C and the fluorescence intensity was measured over a period of 6 days using a Cary Exclipse Fluorometer (Cary, Varian). The

fluorescence intensities were normalized to a Rhodamine B solution in order to account for instrumental and external factors. The fluorescence intensity on Day 0 was taken to be 100%.

Cell Viability Assays: The cellular viability of the U87, 3T3 cells and hMSCs loaded with the ZAIS QDs was assessed using the standard 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. NIH-3T3 cells, U87-EGFRvIII and hMSCs were seeded in a 48-well plate and grown to 70-80% confluency. Various compositions of the ZAIS QDs were mixed with 2.0 μL each of XtremeGene transfection reagent (Roche, USA) to get final concentrations of 25, 50 and 75 μg/mL of the ZAIS QDs. The cells were incubated with these complexes at 37°C in a humidified and 5% CO_2 atmosphere for 6 h, after which they were washed twice with PBS and the media replaced in the transfected wells. Cells treated with only XtemeGene reagent were used as a control. The transfected cells were allowed to grow for 48h, after which the cell viability was assessed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, USA) according to the manufacturer's recommended protocol.

Cellular Imaging using Fluorescence Microscopy: U87 and hMSCs were seeded in a 24-well plate, incubated at 37°C in a humidified and 5% CO_2 atmosphere. After 24 hours, water-soluble MPA-coated ZAIS QD (composition of x=0, y=0.2, Emission of 606 nm) complexed with 2 μL of XtremeGene transfection reagent (at a final concentration of 50 μg/mL QDs) was added to each of the well. The cells were incubated with the QD complex for 6 hours after with the media was replaced. The cells were then allowed to grow for another 24 hours at the same conditions, after which the cells were fixed using 10% Formalin and images were taken using a Nikon Ti-Eclipsed Inverted Fluorescence microscope (Nikon Instruments, Each image was captured with different channels and focus. Images were processed and overlapped using the NIS-Elements software (Nikon, USA).

Cellular Transmission Electron Microscopy: U87-EGFRvIII cells and hMSCs were seeded in a 6 cm dish (BD Falcon, USA) and allowed to grow till 70% confluency. The cells were then treated with above mentioned MPA-coated ZAIS QD-XtremeGene complexes (at a final concentration of 50 μg/mL of QDs) for 6h after which they were washed and the media replaced in the dish. The cells were allowed to grow to 90% confluency after which they were trypsinized and subsequently fixed using Trumps Fixative (Electron Microscopy Sciences, Hatfield, PA, USA) for 2h. The cells were washed twice with sodium cacodylate buffer (pH=7.4, EM Sciences, PA, USA) and post-fixed with 1% aqueous Osmium Tetroxide (Acros Organics, USA) for 1 h. The cells were dehydrated using a graded ethanol washing (50%, 75%, 80%, 95% and 100%) and finally embedded in epoxy resin using the Low Viscosity Spurr's Kit (Electron Microscopy Sciences, Hatfield, PA) according to manufacturer's recommended protocol. The cells were then imaged using a using a JEOL 100CX TEM instrument operating at 80 keV accelerating voltage.

Attachment of siRNA to the MPA-coated ZAIS QDs: For the siRNA to silence EGFP gene in U87-EGFP cells, we utilized the layer-by-layer (LbL) method with slight modifications to the reported procedure.[12] Polyethyleneimine (PEI, MW 10,000) in distilled water was added to MPA-coated ZAIS QDs (composition of x=0, y=0.2, Emission of 606 nm, 400 μl, 10 mg/mL) at a final concentration of 1.0 mg/mL. After sonication for 2-4 hours under neutral pH, the coated particles were purified using ultracentrifugation and subsequent washing with distilled water. After characterization of the surface charge using zeta potential measurement (see Fig. S5), 100 nmol siRNA solution (Integrated DNA Technologies Inc., IA, USA) in PBS was added to 300 μl of PEI coated FeCo/C solution (50 μg/ml). After mild stirring for 1-2 hours, the final product was purified by ultracentrifugation (14,000 rpm) and washed with 1.0 mM NaCl solution. Surface charge was characterized using zeta potential measurement

(Zetasizer Nano ZS, Malvern Instruments, Westborough, MA) to perform the siRNA transfection experiment.

Culture of human U87-EGFP cells for siRNA knockdown experiments: The EGFP overexpressed U87 glioblastoma cells (U87-EGFP) were cultured using DMEM with high glucose, 10% fetal bovine serum (FBS, Gemini Bioproducts), 1% Streptomycin-penicillin and 1% Glutamax (Invitrogen, Carlsbad, CA). Geneticin G418 (100 μg/ml, Invitrogen) was used as a selection marker for the U87-EGFP cells. All cells were maintained at 37 ^{o} C in humidified 5% CO_2 atmosphere. For the knockdown experiments, 10^4 cells were grown to 50-60% confluency in a 48-well plate. The ZAIS QD-MPA-PEI-siRNA conjugates were dispersed in the transfection media (Opti-MEM) at a final concentration of 50 μg/ml. 100 μl of the nanoparticle solution was added to the U87-EGFP cells. U87-EGFP cells treated with only PEI-coated ZAIS QDs were used as a control. After 6 hours of incubation, the solution in each well was removed and exchanged with the growth medium. The cells were allowed to grow for 72 hours after which the GFP knockdown and QD localization was assessed using fluorescence microscopy (Nikon Ti-Eclipsed Inverted Fluorescence microscope, Nikon Instruments, USA). Each image was captured with different channels and focus. Images were processed and overlapped using the NIS-Elements software (Nikon, USA).

Cytotoxicity assessment of ZAIS QDs in oxidative environment: 200 μg/ml solutions of the ZAIS QD (composition of x=0, y=0.2, λ_{em} = 606 nm) in PBS were placed under a longwave (365 nm) UV lamp for 1, 2 and 4 hours. The spherical irradiation was 12 mW/cm^2 as determined with a radiometer. The photo-oxidized ZAIS QDs were then incubated with U87-EGFRvIII cells (10^4 cells seeded in a 48-well plate and grown to 70% confluency) for 48 h, after which the cell viability was assessed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, USA) according to the manufacturer's recommended protocol.

Supporting Figures

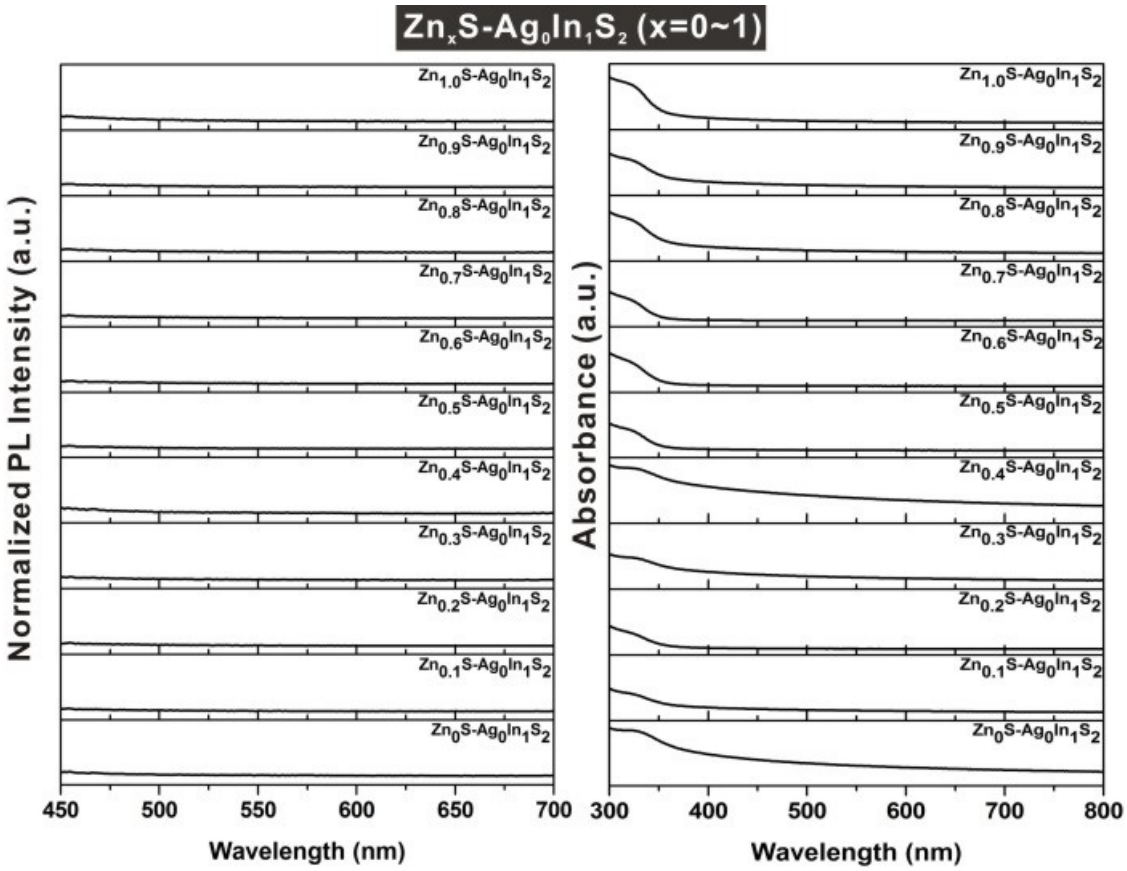

Figure S1a. Absorbance and PL spectra of Zn_xS-$Ag_{0.0}In_{1.0}S_2$

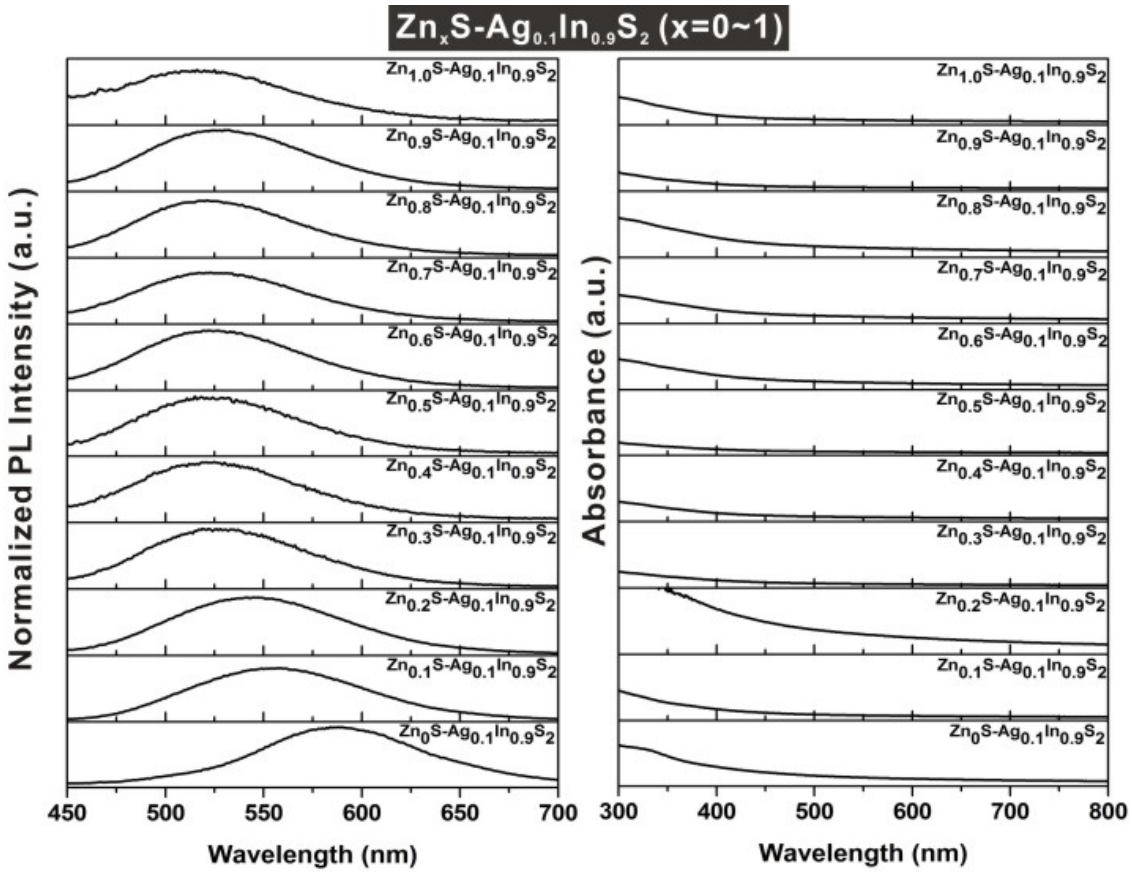

Figure S1b. Absorbance and PL spectra of Zn_xS-$Ag_{0.1}In_{0.9}S_2$

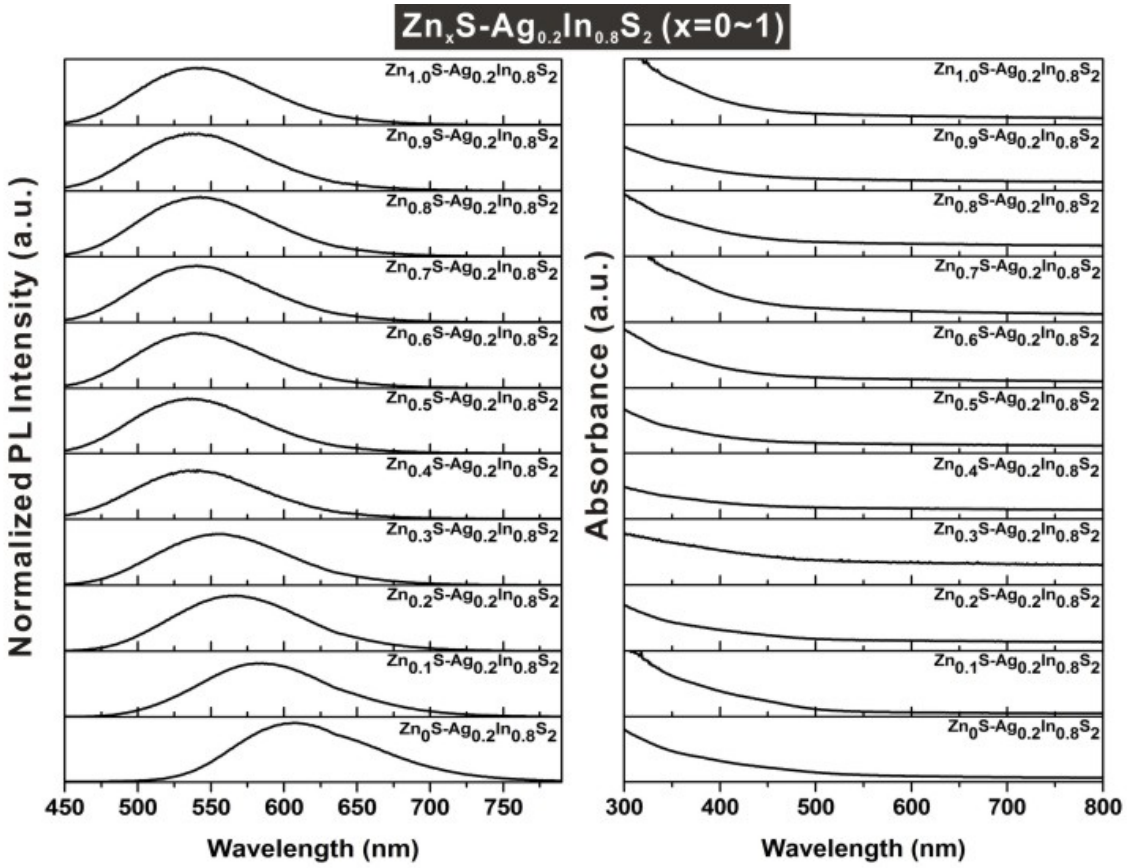

Figure S1c. Absorbance and PL spectra of Zn_xS-$Ag_{0.2}In_{0.8}S_2$

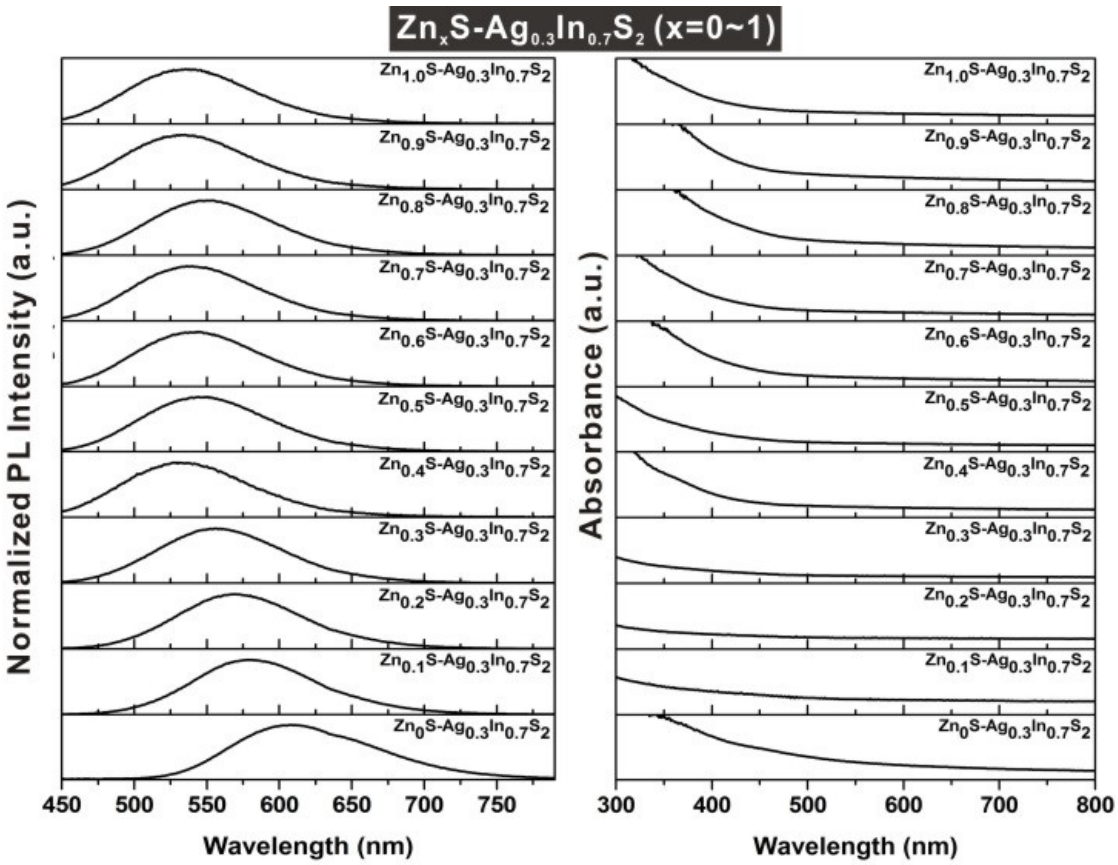

Figure S1d. Absorbance and PL spectra of Zn_xS-$Ag_{0.3}In_{0.7}S_2$

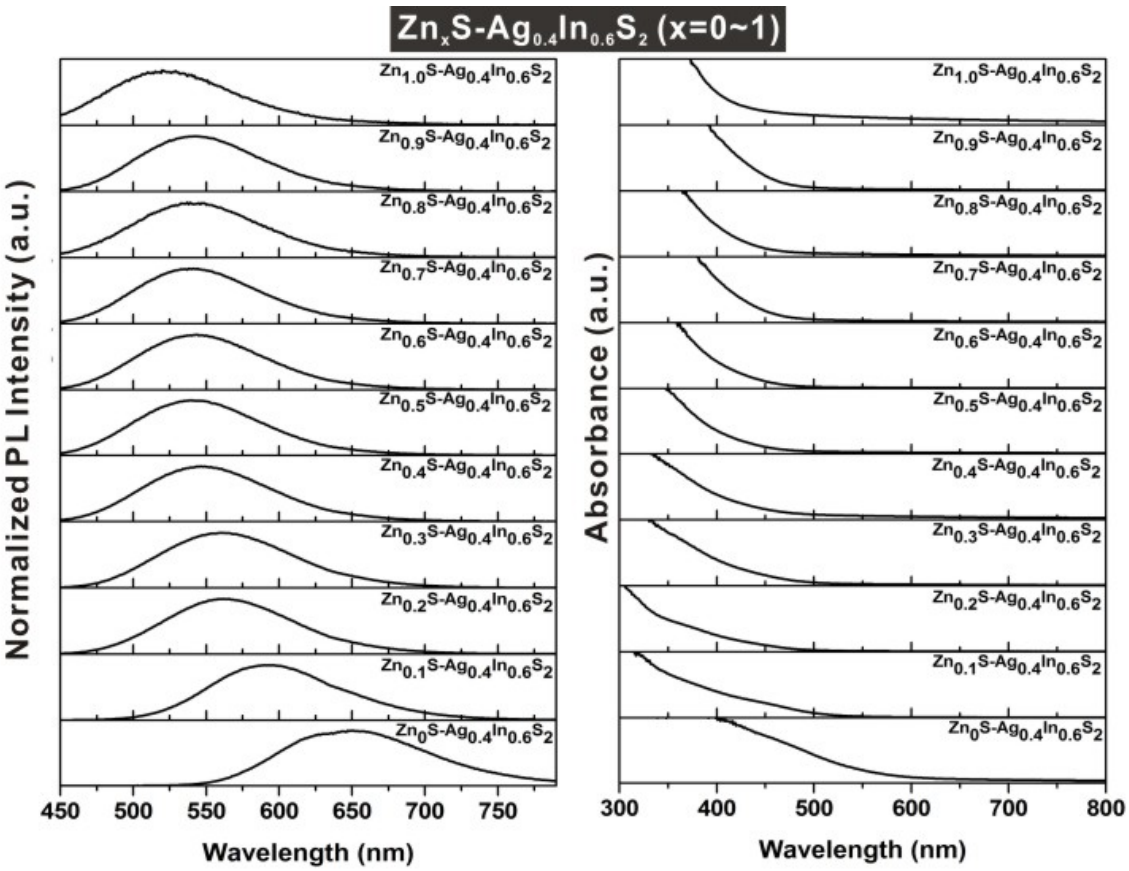

Figure S1e. Absorbance and PL spectra of Zn_xS-$Ag_{0.4}In_{0.6}S_2$

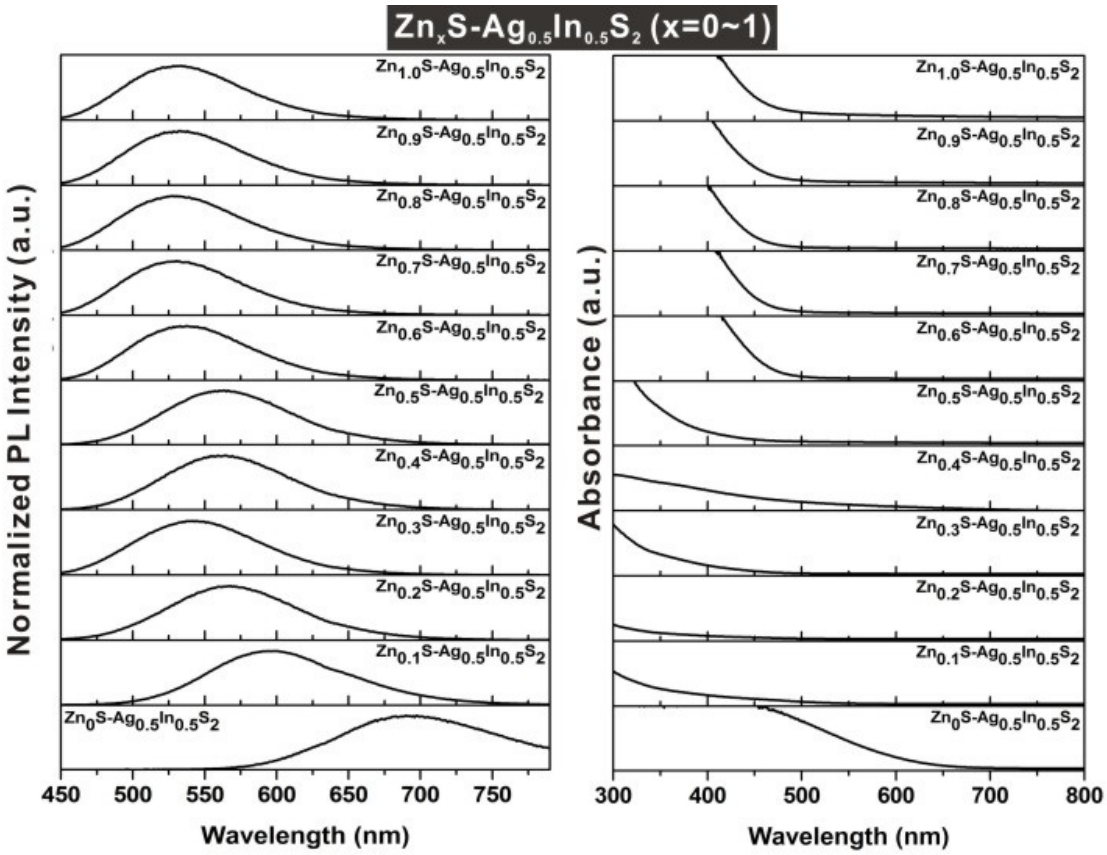

Figure S1f. Absorbance and PL spectra of Zn_xS-$Ag_{0.5}In_{0.5}S_2$

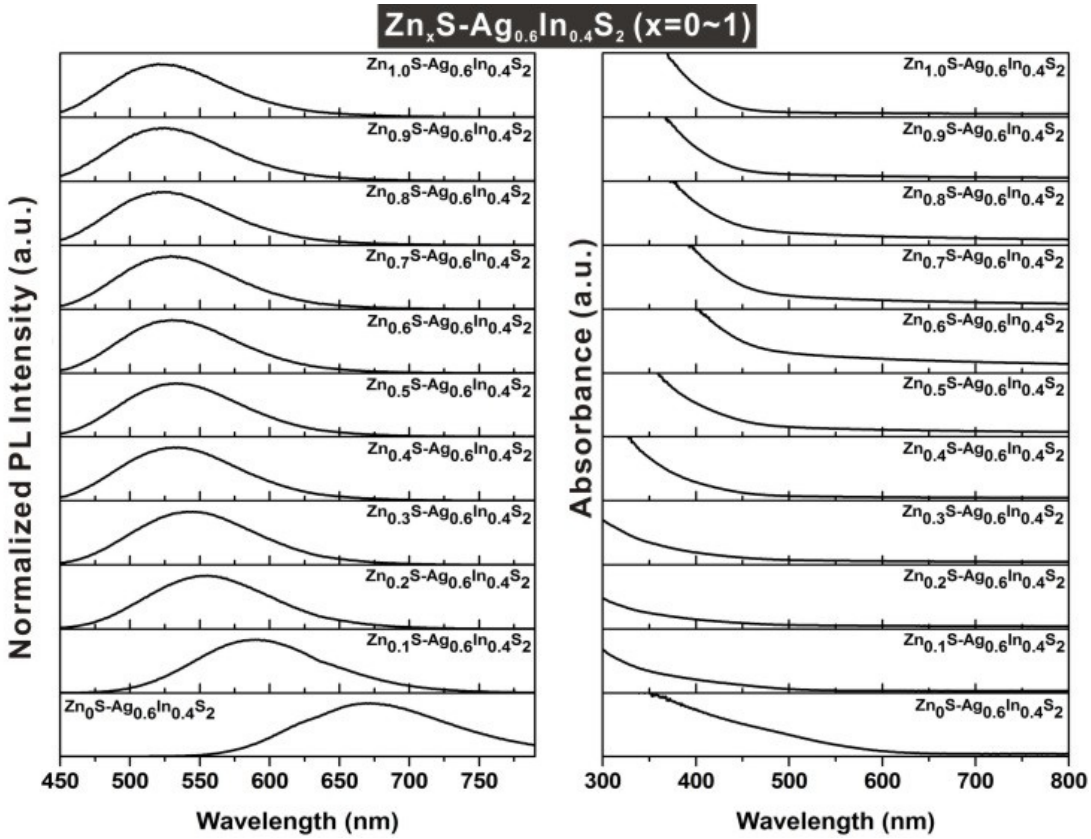

Figure S1g. Absorbance and PL spectra of Zn_xS-$Ag_{0.6}In_{0.4}S_2$

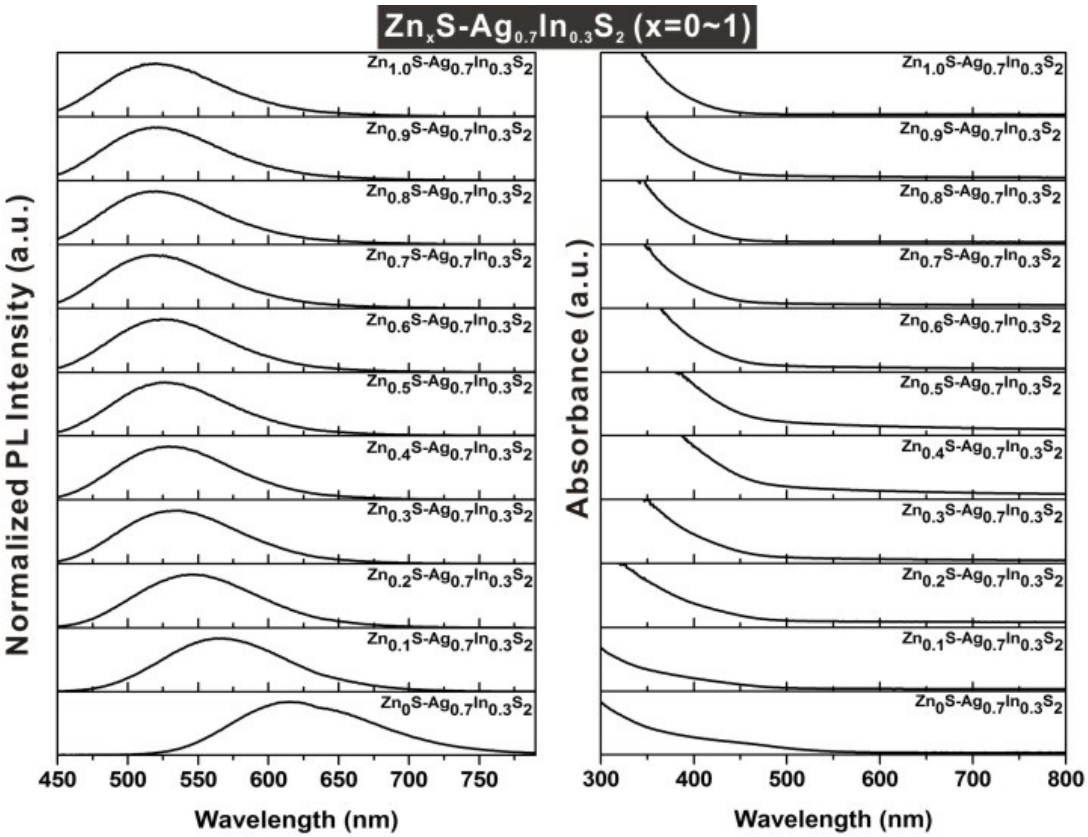

Figure S1h. Absorbance and PL spectra of $Zn_xS\text{-}Ag_{0.7}In_{0.3}S_2$

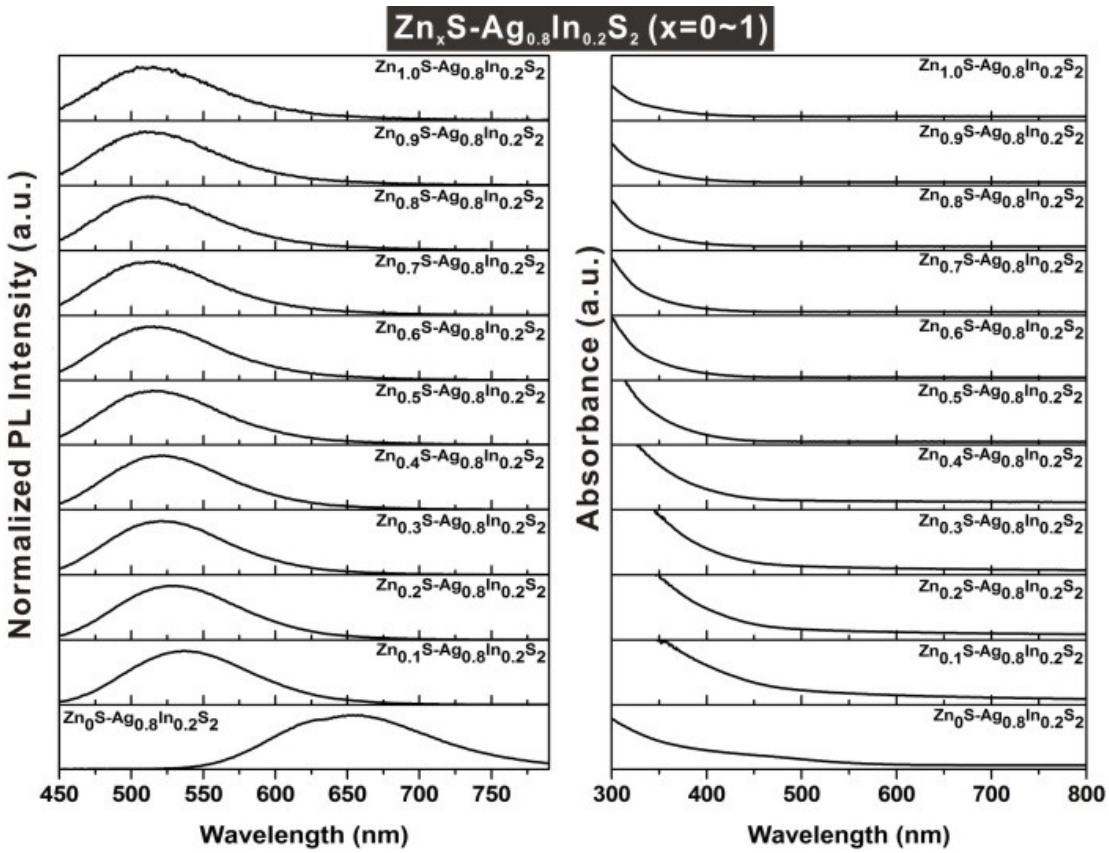

Figure S1i. Absorbance and PL spectra of Zn_xS-$Ag_{0.8}In_{0.2}S_2$

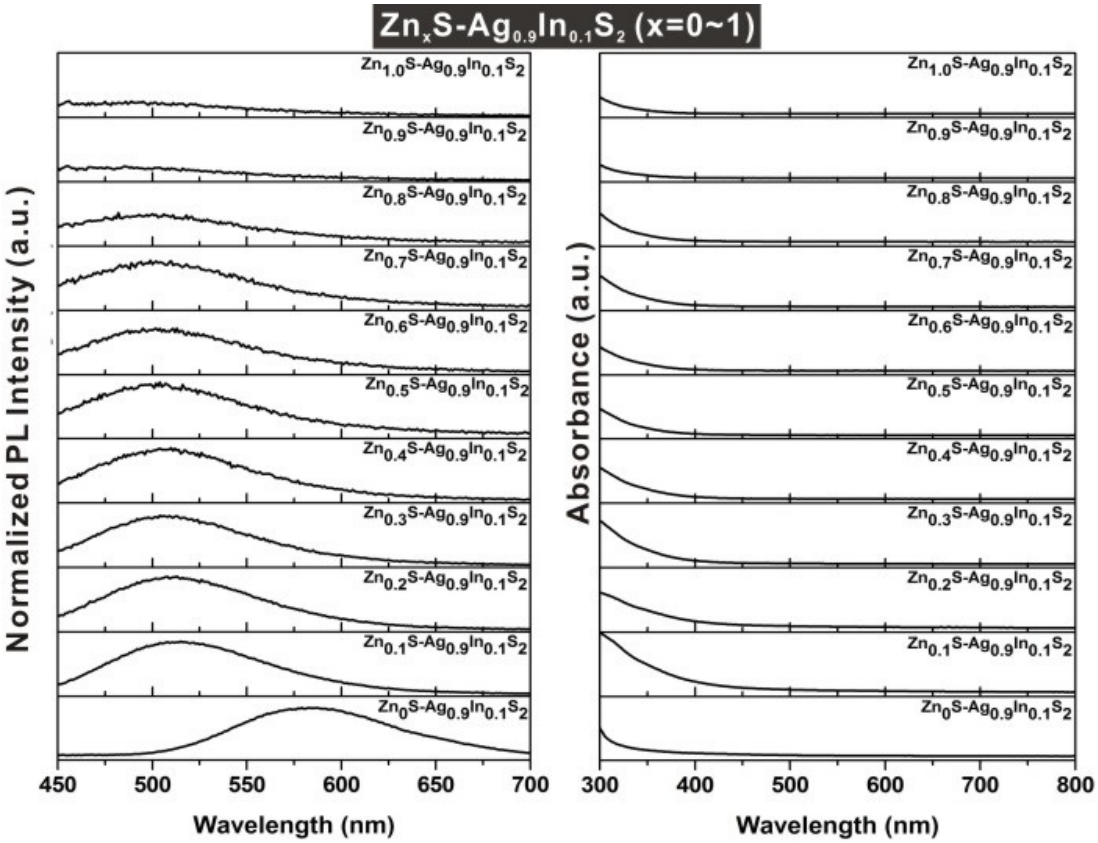

Figure S1j. Absorbance and PL spectra of Zn_xS-$Ag_{0.9}In_{0.1}S_2$

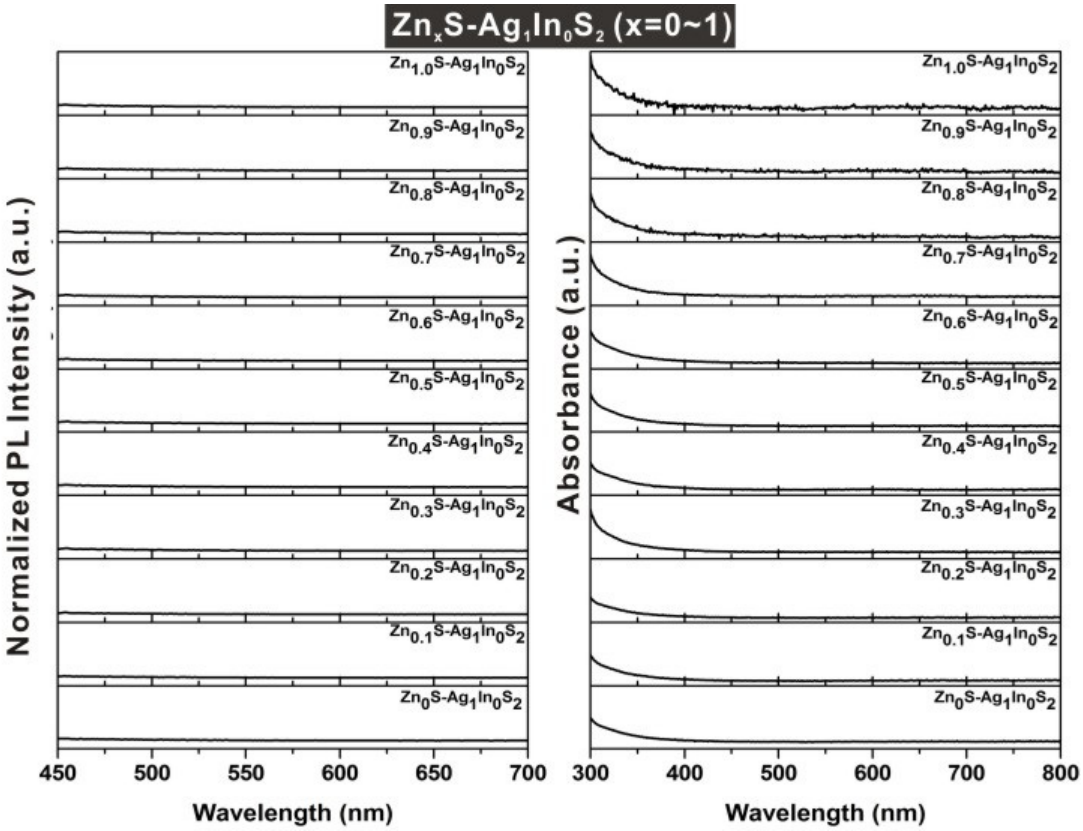

Figure S1k. Absorbance and PL spectra of Zn_xS-$Ag_{1.0}In_0S_2$

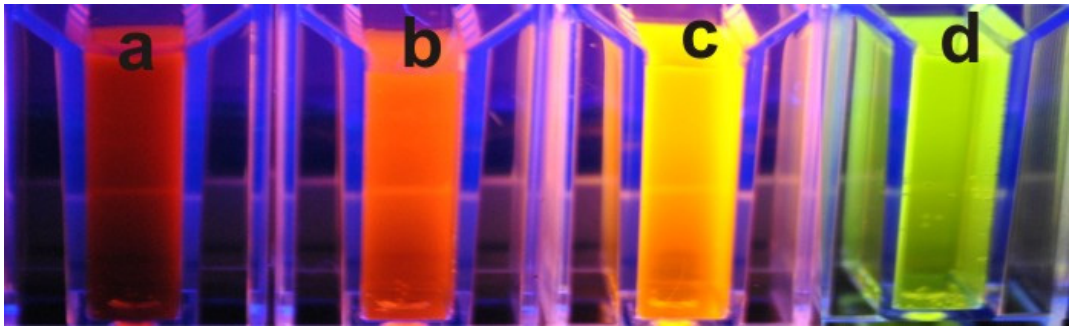

Figure S2. Fluorescence image of the ZAIS QDs depicting the PL stability of the QDs after 2 months of storage. **(a)** Zn_0S-$Ag_{0.5}In_{0.5}S_2$ **(b)** Zn_0S-$Ag_{0.2}In_{0.8}S_2$ **(c)** $Zn_{0.3}S$-$Ag_{0.4}In_{0.6}S_2$ and **(d)** $Zn_{0.6}S$-$Ag_{0.5}In_{0.5}S_2$

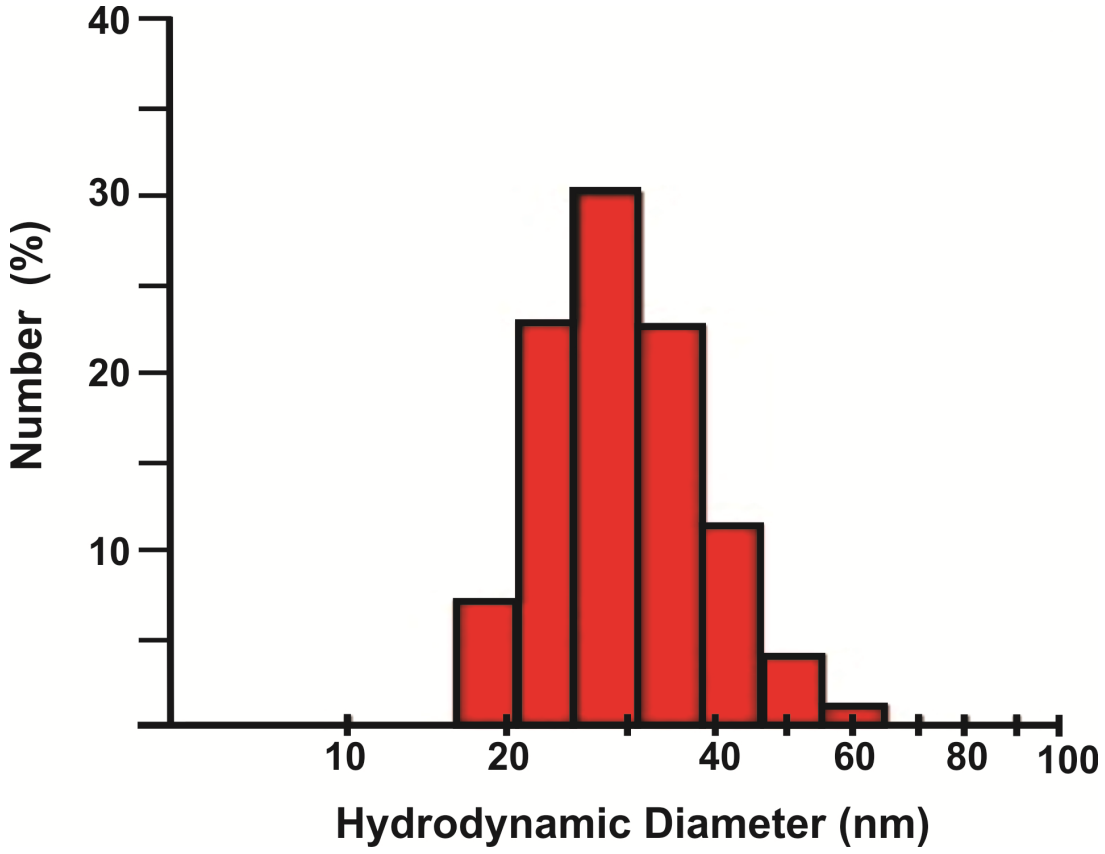

Figure S3. Size distribution of the ZAIS QD ($Zn_0SAg_{0.2}In_{0.8}S_2$) as determined by dynamic light scattering. The average size of the QDs in water was around 30nm with a polydispersity index (PDI) value of 0.302 suggesting that the ZAIS QDs were quite monodisperse

Sample No.	ZAIS QD composition	Emission Wavelength (nm)	Quantum Yield relative to CdSe/ZnS QD (%)
1	Zn_0S-$Ag_{0.5}In_{0.5}S_2$	720	57.6
2	Zn_0S-$Ag_{0.2}In_{0.8}S_2$	606	336
3	Zn_0S-$Ag_{0.5}In_{0.5}S_2$	538	241

Figure S4. PL quantum yield measurements of the ZAIS QDs as compared CdSe/ZnS QDs. QYs of the ZAIS QD solutions were determined by comparing the integrated emission from the nanocrystals to Rhodamine B and Rhodamine-6G dye solutions of matched absorbances. Samples were diluted so that they were optically thin. The QY values are reported as percentge values relative to the CdSe/ZnS quantum yield which was found to have a value of 0.4

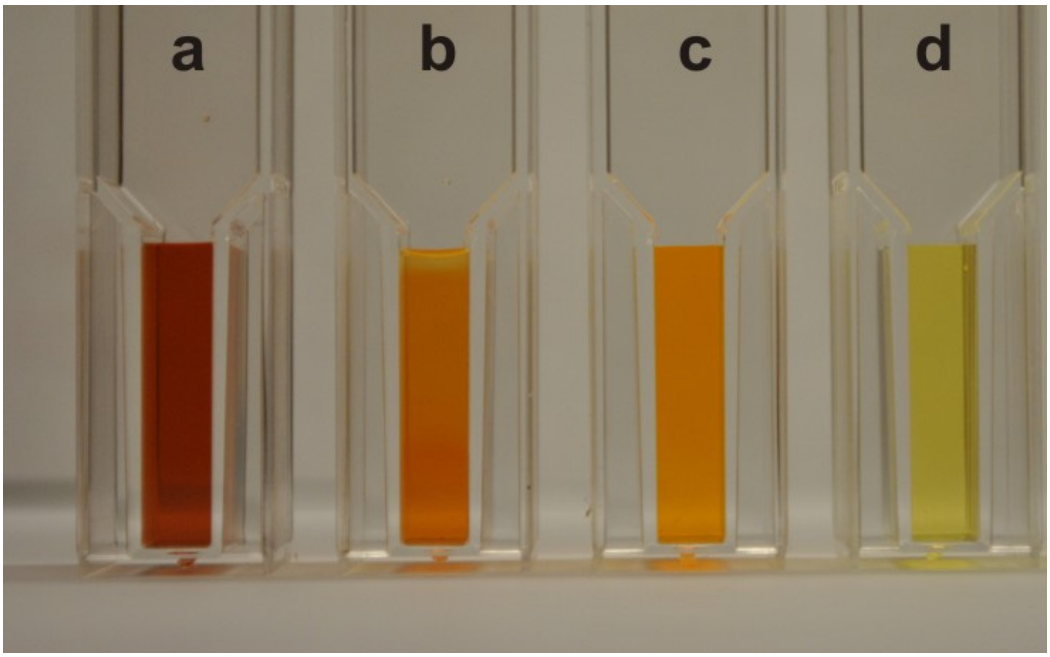

Figure S5. Image depicting the colloidal stability of the MPA-coated ZAIS QDs at physiological conditions. **(a)** Zn_0S-$Ag_{0.5}In_{0.5}S_2$ **(b)** Zn_0S-$Ag_{0.2}In_{0.8}S_2$ **(c)** $Zn_{0.3}S$-$Ag_{0.4}In_{0.6}S_2$ and **(d)** $Zn_{0.6}S$-$Ag_{0.5}In_{0.5}S_2$. The image shows that the water-soluble ZAIS QDs were very stable at physiological conditions (in PBS buffer, pH=7.4), without any sign of aggregation even after several months of storage.

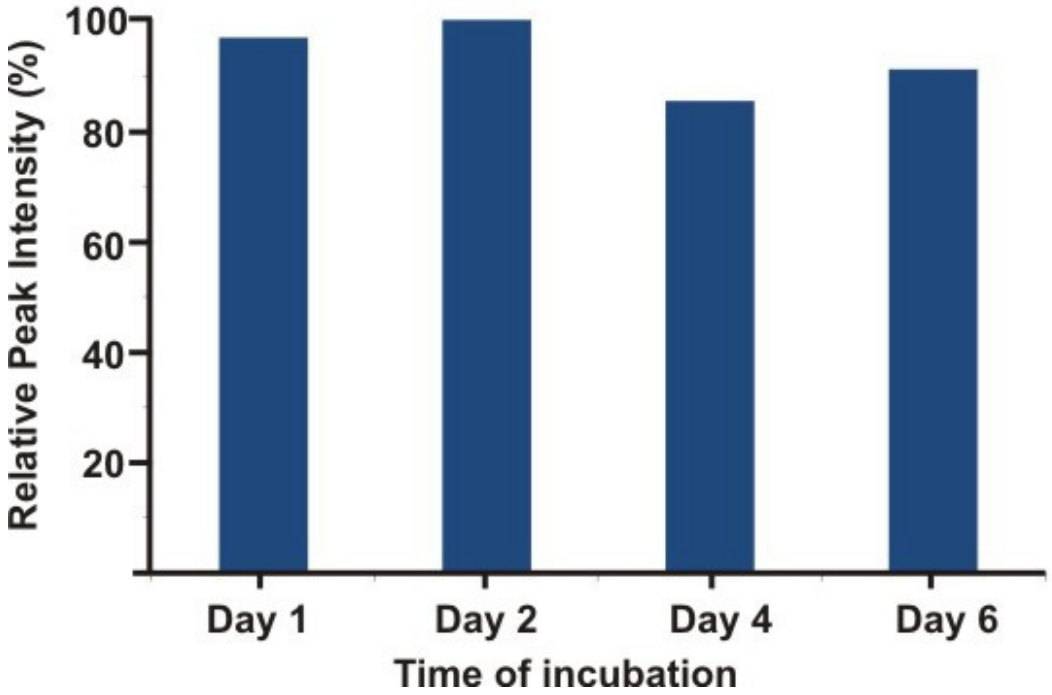

Figure S6. PL stability of ZAIS QD in PBS buffer at 37 ºC. The PL stability of the MPA-coated Zn_0S-$Ag_{0.2}In_{0.8}S_2$ QDs was tested in phosphate buffered saline (PBS, pH=7.4) at 37 ºC over period of 6 days. The PL peak intensities are reported as percentages relative to that on Day 0.

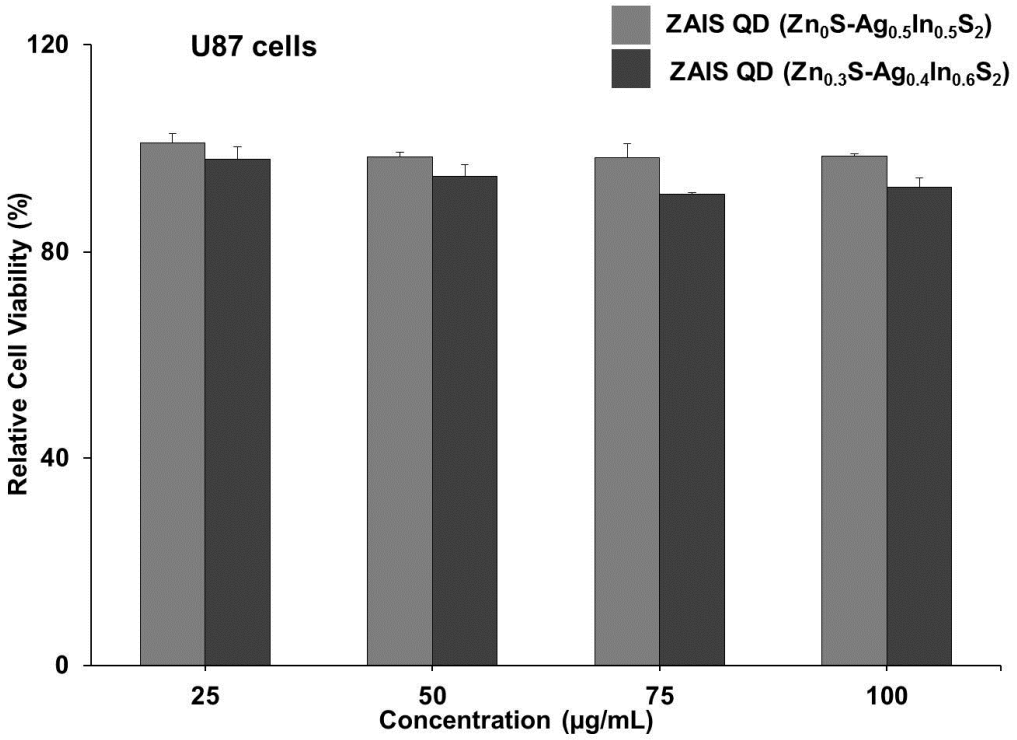

Figure S7. Cellular cytotoxicity of select ZAIS QD compositions in U87 brain cancer cells. The QD composition is indicated in the figure. The results are presented as means ± standard deviation from three separate experiments.

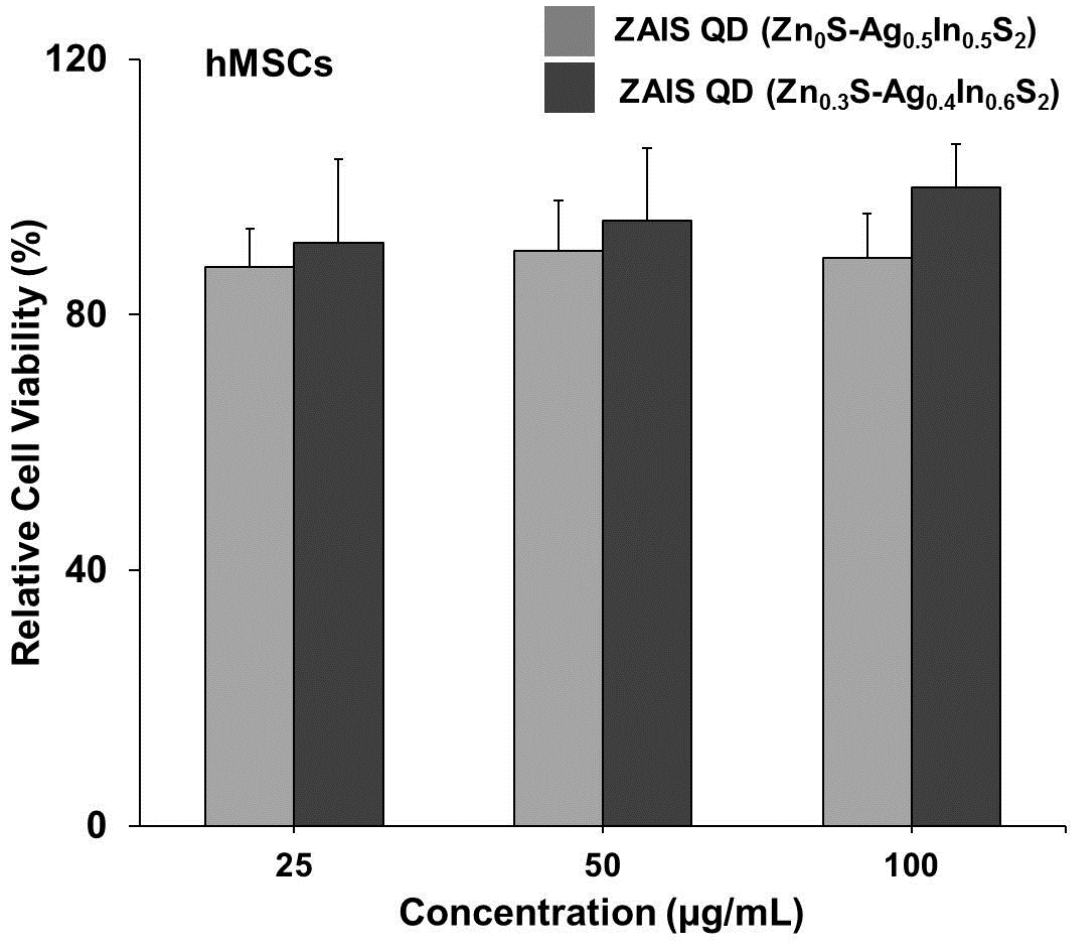

Figure S8. Cellular cytotoxicity of select ZAIS QD compositions in human mesenchymal stem cells. The QD composition is indicated in the figure. The results are presented as means ± standard deviation from three separate experiments.

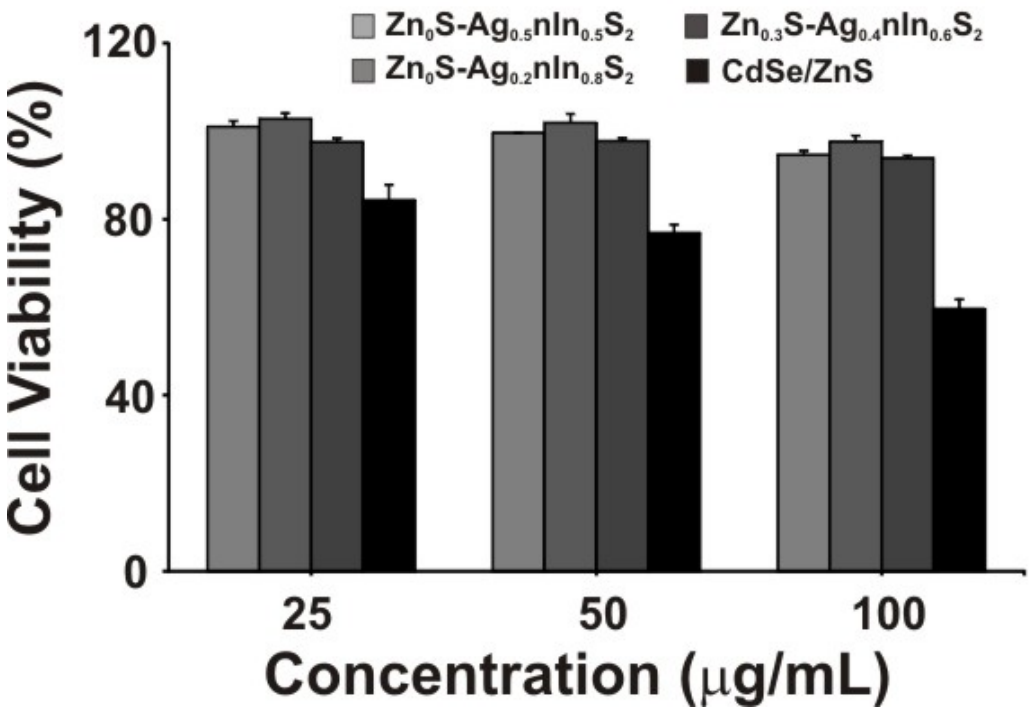

Figure S9. Cellular cytotoxicity of select ZAIS QD compositions in normal (NIH-3T3 mouse fibroblasts) cells. The QD composition is indicated in the figure. The results are presented as means ± standard deviation from three separate experiments.

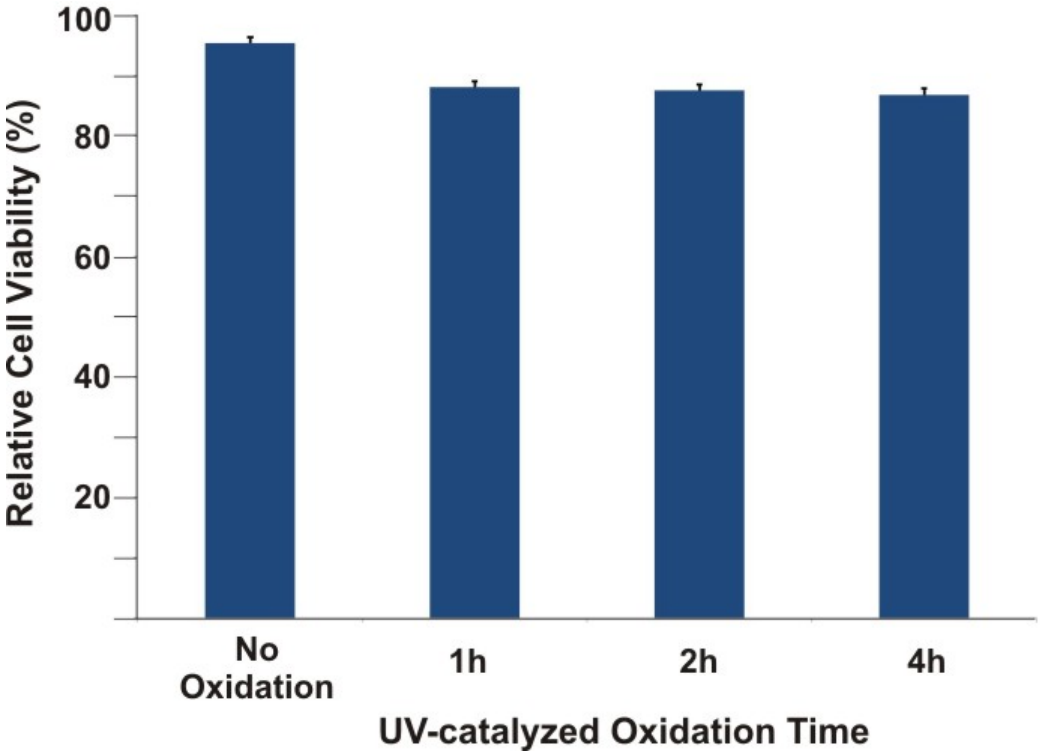

Figure S10. Cellular cytotoxicity of water-soluble ZAIS QD in an oxidative environment. The effect of oxidation, induced by high-energy UV irradiation, on the toxicity of select water soluble ZAIS QD (x=0, y=0.2) was tested in U87 glioblastoma cells. The ZAIS QD solutions (200 µg/mL) were found to be non-toxic even after 4 hours of UV-catalyzed surface oxidation. The viabilities were normalized to non-treated controls (No UV and no QDs) . The results are presented as means ± standard deviation from three separate experiments.

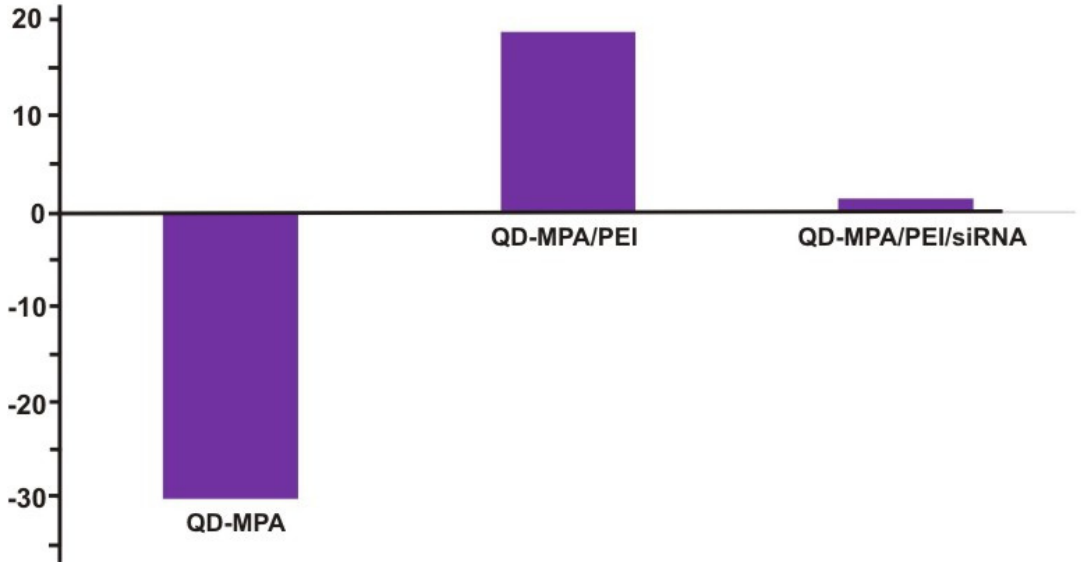

Figure S11. Zeta potential measurements of ZAIS QD-MPA-PEI-siRNA to ensure appropriate layer-by-layer coating. The zeta potential value increased from -30.1 mV to 18.9 mv after the coating of a single layer of PEI on the MPA-coated ZAIS QDs. Upon complexation of negatively charged siRNA molecules, the zeta potential value dropped to 1.4 mv. Since the zeta potential was still positive after complexation of siRNA, coating with a second layer of PEI was avoided.