Supporting information

Quantum Dots in a Amphiphilic Polyethyleneimine Derivative Platform for Cellular Labeling, Targeting, Gene Delivery, and Ratiometric Oxygen Sensing

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Experimental details

Materials and instruments:

Cadmium acetate dehydrates (99.999%), oleic acid (tech, 90%), selenium shots (99.99%), trioctylphosphine (tech, 90%, TOP), oleylamine (tech, 70%), cadmium oxide (99.5%), zinc oxide (99.0%), octadecene (tech, 90%), sulfur (powder, 99.5%), hexadecyltrimethylammonium bromide (CTAB, 98%), tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) bis(hexafluorophosphate) complex (>95.0%) were purchased from Sigma Aldrich. Branched polyethyleneimine (PEI) with molecular weight 25K, 750K were purchased from Sigma Aldrich and 70K from Alfa Aesar. RPMI, DMEM, penicillin/streptomycin, fetal bovine serum (FBS), and phosphate buffered saline (PBS) were purchased from Hyclone. Trypsin-EDTA (0.25%) was purchased from GIBCO. 2-(N-morpholino)ethanesulfonic acid (MES) was purchased from USB Corporation. For measuring the cell viability of HeLa or HCT116 cells, cell counting kit-8 (CCK-8, WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] reagent) was purchased from Dojindo Molecular Technologies. siRNA for GFP silencing, lipofectamine, and CellTrace Carboxyfluorescein diacetate succinimidyl ester (CFSE) fluorescent dyes were purchased from Life Technologies. Deionized water (D.I. water) was triply distilled using Millipore filtration system. IR spectra were measured using a Bruker VERTEX 70 FT-IR spectrometer. UV–vis absorption spectra were obtained using an Agilent 8453. Photoluminescence(PL) spectra were obtained using HORIBA FluoroLog. Hydrodynamic (HD) diameter of nanoparticles were measured by Malvern Zetasizer S. Zeta potential of nanoparticle were measured by Malvern Zetasizer Z. Transmission and PL images were recorded using Zeiss Axioplan 2 microscope with 10 x or 40 x objective. The pictures were taken using Zeiss Axiocam HR CCD camera. The spectrum from microscope

images were extracted by optical fiber with Ocean optics spectrometer. Confocal microscope images were acquired with an Olympus FV-1000 confocal laser scanning microscope using a 488 nm CW He–Ne laser as the excitation source. The laser beam was focused by a 60× objective and the images were recorded with a 550–650 nm band pass filter to visualize the PL signal from the QDs. The distributions of the QDs inside the cells were obtained in the z-axis cross-sectional scan mode. For myoglobin detection using conventional ELISA, the absorbance was measured using a Multiskan EX microplate spectrophotometer. For flow cytometry measurements, QD labeled cells were detected and quantified by FACS Calibur (BD bioscience).

Methods

Synthesis of CdSe/CdS/ZnS (core/shell/shell) quantum dots

CdSe bare nanocrystals were made by the following procedure. For cadmium precursor, cadmium acetate (1.2 mmol) was dissolved in oleic acid (6.0 mmol) at 100 °C under vacuum. After the solution was cooled to room temperature, the cadmium precursor was mixed with selenium precursor. The selenium precursor was previously prepared by dissolving selenium shots (6.0 mmol) in TOP (6 mL) in a glovebox. ODE (40 mL) and oleylamine (6 mmol) were loaded into a three-neck flask and heated to 300 °C under nitrogen gas flow. At this temperature, the mixture of cadmium and selenium precursors was quickly injected into the reaction flask and the temperature is maintained at 280 °C. The reaction mixture was kept stirring until desired size of CdSe nanocrystals was obtained. Upon completion, the mixture was cooled to room temperature and diluted by hexanes. For purification, the product mixture was precipitated by excess methanol, collected by centrifugation, and re-dispersed to a small

amount of hexanes. Over-coating of CdS and ZnS shells to obtain CdSe/CdS/ZnS (core/shell/shell) QDs, slightly modified successive ionic layer adsorption and reaction approach was used. 20 ml of ODE, 7 ml of oleylamine, and 500 nmol of CdSe core QDs were loaded in a three-neck flask and following 1 hour of degassing under vacuum, it was heated to 230 °C under nitrogen gas flow. 0.1 M elemental sulfur dissolved in ODE, 0.1 M Cd-oleate in ODE and 0.1 M Zn-oleate were used as precursors for the growth of shell layers. The quantity of precursors used for each monolayer of shells (3 monolayers for CdS shell and 3 monolayers for ZnS shell) were calculated for the successive increases in particle volume as a function of increasing shell thickness. The reaction temperature was 230 °C and growth time was 10 minutes between each addition. The reaction mixture was cooled to room temperature, and diluted with hexanes. The product CdSe/CdS/ZnS (Core/Shell/Shell) QDs were purified using the same methods for the purification of CdSe core QDs.

Synthesis of CdS/ZnS (core/shell) Quantum Dots

CdS bare nanocrystals were made by the following procedure from published elsewhere.[1] For cadmium precursor, cadmium oxide (0.2 mmol) was dissolved in oleic acid (1.6 mmol) and octadecene (ODE, 7.5 mL) at 100 ^{o}C under vacuum. Sulfur precursor (0.1 M) was prepared by dissolving elemental sulfur powder (0.1 mmol) in octadecene (1 mL) at 100 ^{o}C under vacuum. The cadmium precursor solution was loaded into a three-neck flask and heated to 240 °C under nitrogen gas flow. At this temperature, the sulfur precursor solution was quickly injected into the reaction flask and the temperature is maintained at 240 °C for 30 min. The reaction mixture was kept stirring until desired size of CdS nanocrystals was obtained. Upon completion, the mixture was cooled to room temperature and diluted by hexanes. For purification, the product mixture was precipitated by excess methanol, collected

by centrifugation, and re-dispersed to a small amount of hexanes. Over-coating of ZnS shells to obtain CdS/ZnS (core/shell) QDs, slightly modified successive ionic layer adsorption and reaction approach was used. 10 ml of ODE, 2 ml of oleylamine, and 100 nmol of CdS core QDs were loaded in a three-neck flask and following 1 hour of degassing under vacuum, it was heated to 230 °C under nitrogen gas flow. 0.1 M elemental sulfur dissolved in ODE, 0.1 M zinc oleate in ODE were used as precursors for the growth of shell layers. The quantity of precursors used for each monolayer of shells (6 monolayers for ZnS shell) were calculated for the successive increases in particle volume as a function of increasing shell thickness. The reaction temperature was 230 °C and growth time was 10 minutes between each addition. The reaction mixture was cooled to room temperature, and diluted with hexanes. The product CdS/ZnS (core/shell) QDs were purified using the same methods for the purification of CdS core QDs.

Preparations of QD-amPEI with different PEI molecular weight, degree of modification, and mixing ratio between amPEI and QD

Prior to alkyl chain conjugation, water in the 70K polyethyleneimine (PEI) aqueous solution was completely dried out using rotary evaporator at 70 ^{o}C and was dispersed in anhydrous chloroform to be each 0.1 mM solution (The 70K PEI has approximately 1630 amine functional groups.) in three different vials. To the PEI solution, 408, 815, or 1224 molar equivalent of octylisocyanates (for 25, 50, or 75% DOM) were added the three different vials and kept stirred vigorously for one hour. For alkylation of 25K PEI, similar methods was used except molar equivalent of octylisocyanates (145, 290, or 580 molar equivalent of octylisocyanates added for 25, 50, or 75% DOM). For unmodified PEI control sample, PEI itself was used for encapsulation QDs. The QD hexanes solution prepared by the

solvothermal method was purified by precipitation with methanol and re-dispersion in chloroform. For the 610 nm emitting QDs, the concentration of QD solution was spectrophotometrically determined using the extinction coefficient of 2.26×10^6 $cm^{-1} M^{-1}$ at 350 nm. The amPEI solutions in chloroform was mixed with 1 nmol of QDs in chloroform. The mixing ratio between amPEI and QD was varied from 1:10 to 10:1. 10 ml of MES buffer (100 mM, pH 6.5) was added to the QD and amPEI mixture in chloroform and sonicated for 30 minutes. The chloroform in the mixture was removed by vacuum. QD-amPEIs were centrifuged for 20 minutes at 14,000 rpm 4 ^oC, and the excess amPEI polymers were removed by decanting. The spun-down QD-amPEIs were readily dispersible in D.I. water or in buffers for further experiments.

Synthesis of various types of amphiphilic poly(ethyleneimine) (amPEI) polymers (amPEI(70K, C5), amPEI(70K, C8), amPEI(70K, C16), amPEI(25K, C16), and amPEI(750K, C16)) and encapsulation of QDs with those amPEI polymers

Prior to alkyl chain conjugation, water in the 70K PEI aqueous solution was completely dried out using rotary evaporator at 70 ^oC and was dispersed in anhydrous chloroform to be 0.1 mM solution (The 70K PEI has approximately 1630 amine functional groups) in three different vials. To the PEI solution, 815 molar equivalent of alkylisocyanates (AIs, pentyl-, octyl-, or hexadecylisocyanate) were added and kept stirred vigorously for one hour. For alkylation of 25K or 750K PEI, similar procedure was used except molar equivalent of hexadecylisocyanates (290 or 8700 molar equivalent of hexadecylisocyanates added). The QD hexanes solution prepared by the solvothermal method was purified by precipitation with methanol and re-dispersion in chloroform. For the 610 nm emitting QDs, the concentration of QD solution was spectrophotometrically determined using the extinction coefficient of 2.26 ×

10^6 cm^{-1} M^{-1} at 350 nm. The amPEI solutions in chloroform was mixed with 1 nmol of QDs in chloroform. The mixing ratio between amPEI and QD was 10:1. 10 ml of MES buffer (100 mM, pH 6.5) was added to the QD and amPEI mixture in chloroform and sonicated for 30 minutes. The chloroform in the mixture was removed by vacuum. QD-amPEIs were centrifuged for 20 minutes at 14,000 rpm 4 ^oC, and the excess amPEI polymers were removed by decanting. The spun-down QD-amPEIs were readily dispersible in D.I. water or in buffers for further experiments.

Encapsulation of QDs with CTAB, Lipofectamine™

1 nmol of CdSe/CdS/ZnS (core/shell/shell) QDs dispersed in chloroform (10 μM) was used for encapsulation with hexadecyltrimethylammonium bromide (CTAB) and Lipofectamine™. 10^3 equivalent CTAB and Lipofectamine™ to QDs were dissolved in the QD solution in chloroform. Add the 10 mL of MES buffer (10 mM, pH 6) to the CTAB-QD or Lipo-QD and sonicated for 30 minutes. The mixture solution was heated to 60 ^oC and chloroform was removed using vacuum. The samples were used for cellular labeling with no further purification.

Synthesis of (+), (-) QDs, or Lipo-(-)-QDs

Hydrophobic ligands of CdSe/CdS/ZnS (core/shell/shell) quantum dots prepared by solvothermal pyrolysis were exchanged with lipoic acid ((-) ligands) and primary amine decorated lipoic acid ((+) ligands), which were made by the following procedure from published elsewhere.[38] In case of (-) ligands, 10^5 equivalent amounts of (-) or (+) ligands to QDs were dissolved in deionized (D.I.) water (1 mL). Two equivalents of sodium

borohydride to ligands were added to the ligand solution and vigorously stirred for 20 min under N_2 gas flow at room temperature. (-) and (+) ligands were obtained in the aqueous solution. Crude QDs were purified in a MeOH/hexanes anti-solvent/solvent system. The QDs were dispersed in chloroform. The QD solution (1 mL) was added to the ligand solution and further stirred for 2 hours at room temperature. The QDs were transferred from the organic layer to the aqueous layer. To remove excess free ligands, the QD solution was dialyzed using Amicon 50 kDa MW cutoff centrifugal filters. For Lipo-(-)-QD preparation, 10^3 equivalent Lipofectamine to (-)-QDs was added in 1 μM of (-)-QDs solution (dispersed in D.I. water). The samples were used for cellular labeling with no further purification.

GFP siRNA gene delivery experiment using QD-amPEI-siRNA

QD-amPEI-siRNA sample was prepared by mixing GFP siRNA with QD-amPEI composites in DMEM serum free media. For 'QD-amPEI' sample, 10 nM QD-amPEI were co-cultured with MDCK-GFP cells. For 'QD-amPEI-siRNA' sample, 10 nM QD-amPEI-siRNA (electrostatically coupled QD-amPEI with 30 nM GFP siRNA) were co-cultured the cells. For 'siRNA only' sample, 300 nM GFP siRNA were co-cultured the cells. For 'Lipofectamine-siRNA' sample, 1.5 μM Lipofectamine-siRNA (electrostatically coupled 1.5 μM Lipofectamine with 300 nM GFP siRNA) were co-cultured the cells. All of the samples were prepared and treated in serum free media. For 'w/o QD-amPEI (control)' set, only MDCK-GFP cells were incubated in serum free media for 4 hours without any samples. MDCK-GFP transfected cells in 24-well plate with cover-slip (10000 cells per each well) were co-incubated with the 4 different samples. All the samples washed out after 4 hours co-incubation and the cells were additionally incubated for 48 hours to evaluate the siRNA silencing effect. The cells are fixed with 4% formaldehyde for 10 min at room temperature

and washed with PBS buffer (0.1 M, pH 7.4). For microscope imaging, cover slips attached the cells were mounted on glass slides using aqueous mounting medium with an anti-fading agent.

Green fluorescence protein GFP small-interfering RNA (siRNA) sequence

siRNA sequence for GFP silencing:

(antisense): 5'-CAA GCU GAC CCU GAA GUU CTT-3'

(sense): 5'-GAA CUU CAG GGU CAG CUU GTT-3'

Synthesis of hyaluronic acid (HA)-QD-amPEI composites

HA-QD-amPEIs were prepared by mixing sodium hyaluronates to QD-amPEI solution (pH 6.5, 0.1 M MES buffer) with for various mixing ratios (HA:QD-amPEI=1:5000, 1:1000, 1:500, 1:200, 1:100, 1:50, 1:20, 1:10, 1:5, 1:2).

Cellular labeling with HA-QD-amPEIs

Cultured HeLa and human dermal fibroblast (Hdf) cells in 24-well plate with cover-slip (10000 cells per each well) were co-incubated with the HA-QD-amPEI sample, which 50 nM of HA-QD-amPEI samples in complex media (the ratio of MES buffer to DMEM serum free media is 2) for 2 hours. For control sample, these two kinds of cells were co-incubated with QD-amPEI sample or DMEM serum free media only. The cells were washed with PBS buffer

(0.1 M, pH 7.4) for 3 times. The cultured cells were fixed with 4% formaldehyde for 10 min at room temperature and washed with PBS buffer (0.1 M, pH 7.4). For microscope imaging, cover slips attached the cells were mounted on glass slides using aqueous mounting medium with an anti-fading agent.

Synthesis of oxygen sensing probe (QD-Ru-amPEIs)

Ruthenium based oxygen sensing dye (tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) bis(hexafluorophosphate) complex, Ru dye) contained QD-amPEI composites (QD-Ru-amPEIs), QD-Ru-amPEIs, were synthesized as following procedure. CdS/ZnS (core/shell) QDs in hexane was purified using methanol 3 times, dried and re-dissolved in chloroform. Concentration of the QD solution was calculated by absorbance at 350 nm and first band edge peak. Amphiphilic poly(ethyleneimine) (amPEI, hexadecylisocyanate derivatized PEI) polymer solution in chloroform was mixed with 13 nmol of QDs and 50 nmol of Ru dyes in chloroform. The mixing ratio of amPEI to QDs was chosen as 10 for efficient cell labeling. For QD-Ru-amPEI composites preparation, 10 ml of MES buffer (10 mM, pH 6.5) was added to the QD-Ru-amPEI mixture and sonicated for 30 minutes. The chloroform residue was evacuated by vacuum.

Reversible oxygen concentration sensing and oxygen concentration calibration experiment with QD-Ru-amPEIs *in vitro*

1 μM QD-Ru-amPEIs were dissolved in MES buffer were bubbled with ambient air (for normoxic condition) or 100% nitrogen gas (for hypoxic condition) for 10 minutes for each condition, reversibly up to 12 cycles. 1 μM QD-Ru-amPEIs were dissolved in MES buffer

were gas mixtures composed with 0, 2, 5, 10, 15, 20, 40, 60, 80, 100% oxygen/nitrogen gas for 10 minutes for each condition, sequentially. The fluorescence spectrum of QD-Ru-amPEI solutions were measured by spectrofluorometer at certain condition. Phosphorescence intensity ratio from QD-Ru-amPEI was fitted in two-component Stern-Volmer equation.

Oxygen sensing experiment with QD-Ru-amPEIs in cultured cells and spheroid cells

QD-Ru-amPEIs were used for sensing oxygen concentration of cultured HCT116 cells and spheroid HCT116 cells. Cultured HCT116 cells in 24-well plate with cover-slip (10000 cells per each well) were co-incubated with the sample, which 50 nM of QD samples in RPMI serum free for 4 hours. The cells were washed with PBS buffer (0.1 M, pH 7.4) for 3 times and incubated with 100% N_2 purged RPMI serum free media for 4 hours. For spheroid HCT116 cells, spheroid cells were prepared by hanging-drop assay. 10^5 cells were dispersed in 20 μL of RPMI growth media and the solution was dropped underneath the lid of cell culture dish. The Lid of cell culture dish was flipped to place the cells in cell culture dish for 2 days for inducing spheroid formation. The spheroid HCT116 cells were co-incubated with the sample, which 100 nM of QD samples in RPMI serum free for 4 hours. The cells were washed with PBS buffer (0.1 M, pH 7.4) for 3 times. The cultured cells or spheroid cells were fixed with 4% formaldehyde for 10 min at room temperature and washed with PBS buffer (0.1 M, pH 7.4). For microscope imaging, cover slips attached the cells were mounted on glass slides using aqueous mounting medium with an anti-fading agent. Spheroid cells were moved from cell culture dish to glass slide containing the mounting solution. For measuring PL spectrum from the cells, optical fiber connected spectrophotometer was mounted on the C mount connector of microscope.

Cytotoxicity Assay

To determine the cytotoxicity of QD samples, 5000 HeLa cells in 96-well plate were treated with 10 nM of QD samples in RPMI serum free media for 12, 24, 48 hours. And at each time point, 10 μl of CCK-8 reagents (WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] reagent) were added to the cells and co-incubated for 1 hour. The absorbance at 450 nm was measured using a microplate spectrophotometer. For measuring the cell viability of oxygen sensing probes, HCT116 cells were used for measuring the cytotoxicity of QD-Ru-amPEIs with similar procedure above. To determine the cytotoxicity of QD samples, 5000 HeLa cells in 96-well plate were treated with 10, 30, 100 nM of QD-Ru-amPEI in DMEM serum free media for 3, 6, 12, 24 hours. And at each time point, 10 μl of CCK-8 reagents (WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] reagent) were added to the cells and co-incubated for 1 hour. The absorbance at 450 nm was measured using a microplate spectrophotometer.

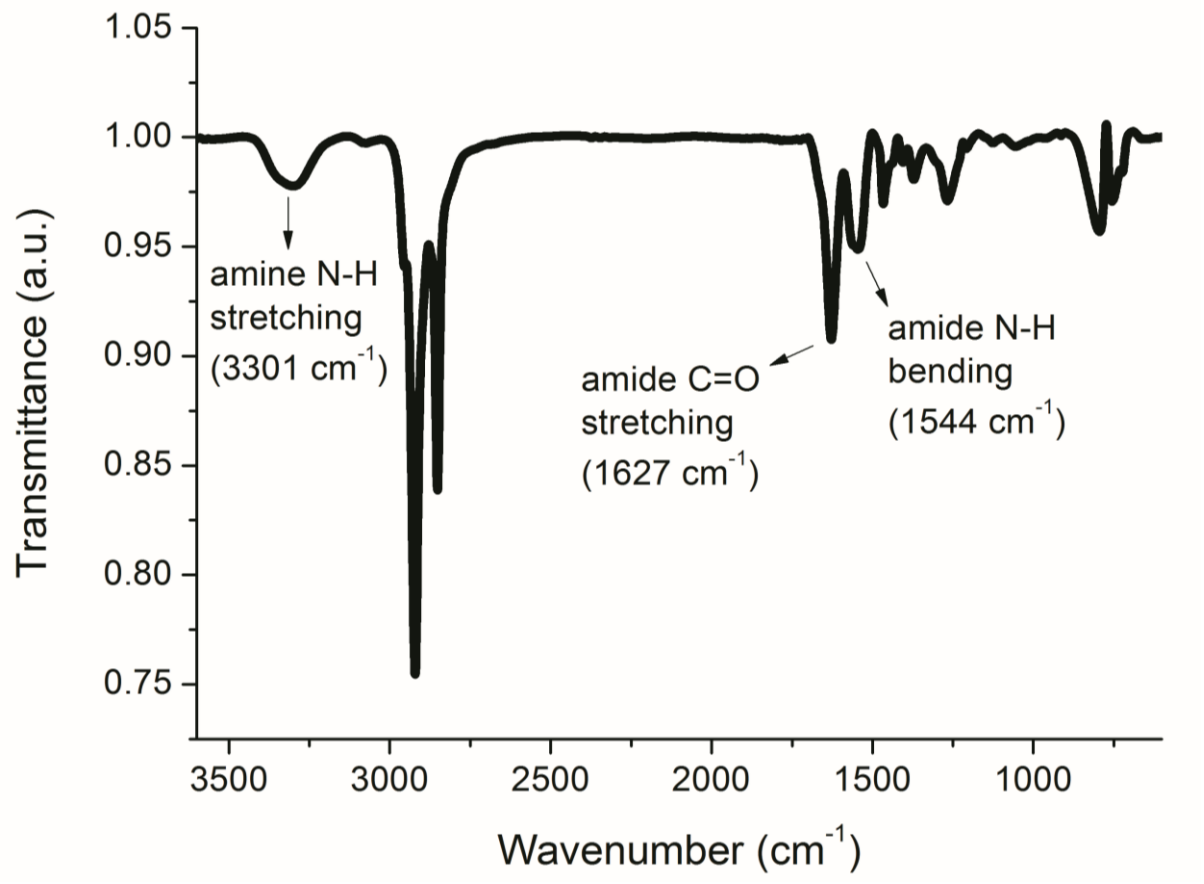

Figure S1. FT-IR characterization for amphiphilic polyethyleneimine. The spectra of hexadecylisocyanate derivatized polyethyleneimine showing by the designating peaks (amide C=O stretching (1627 cm^{-1}) and amide N-H bending (1544 cm^{-1}) from urea bonds, amine N-H stretching (3301 cm^{-1}) from unmodified amine groups).

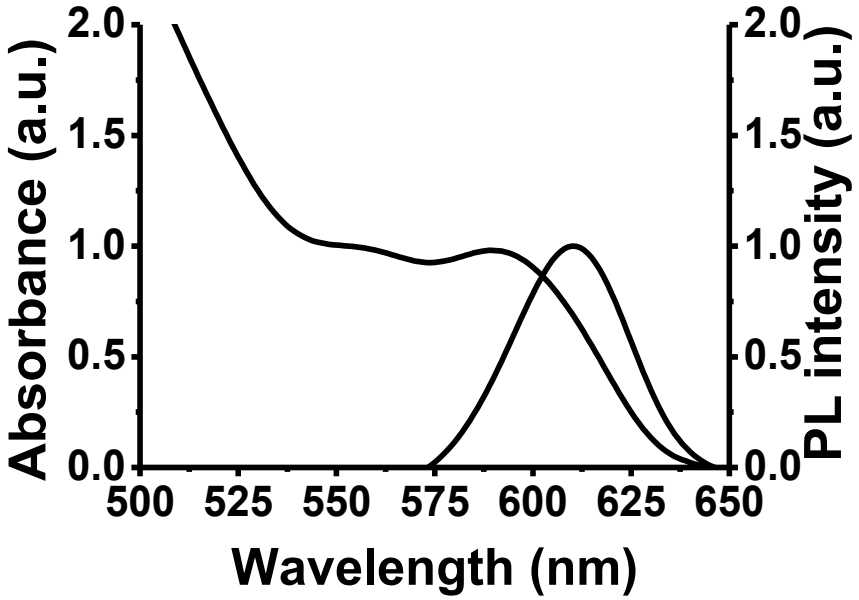

Figure S2. Absorption and emission spectrum of as-synthesized CdSe/CdS/ZnS (core/shell/shell) quantum dots.

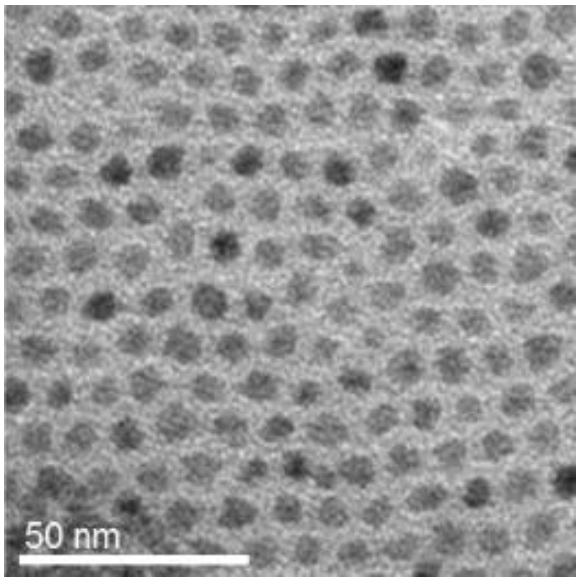

Figure S3. Transmission electron microscopy image of as-synthesized CdSe/CdS/ZnS (core/shell/shell) quantum dots.

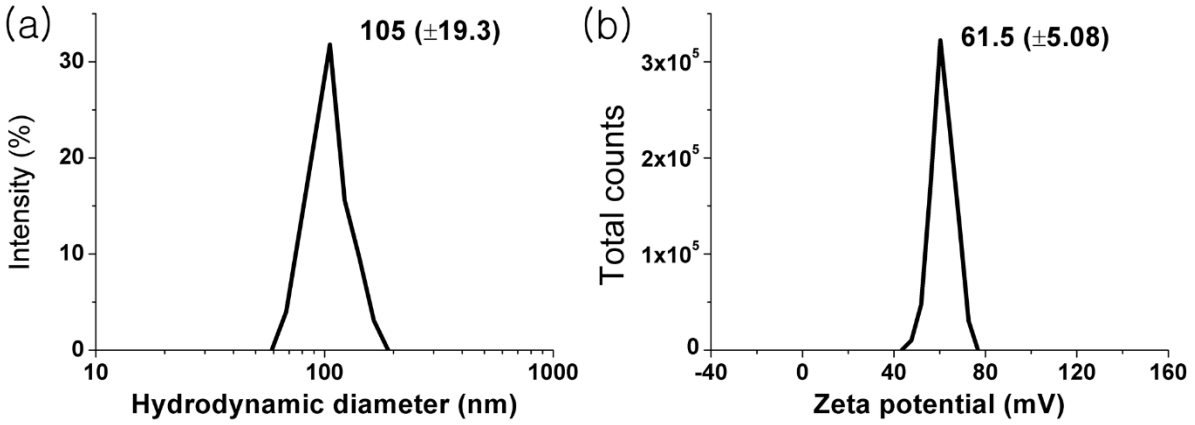

Figure S4. Graphs showing (a) hydrodynamic diameter and (b) zeta potential value of QD-amPEI(70K, C16) (QDs wrapped in the amphiphilic polyethyleneimine (amPEI) polymer vehicle). The QD-amPEI was encapsulated amPEIs with 70K molecular weight of the PEI, 50% of the degree of modification, and 10 mixing ratio of amPEI to QDs.

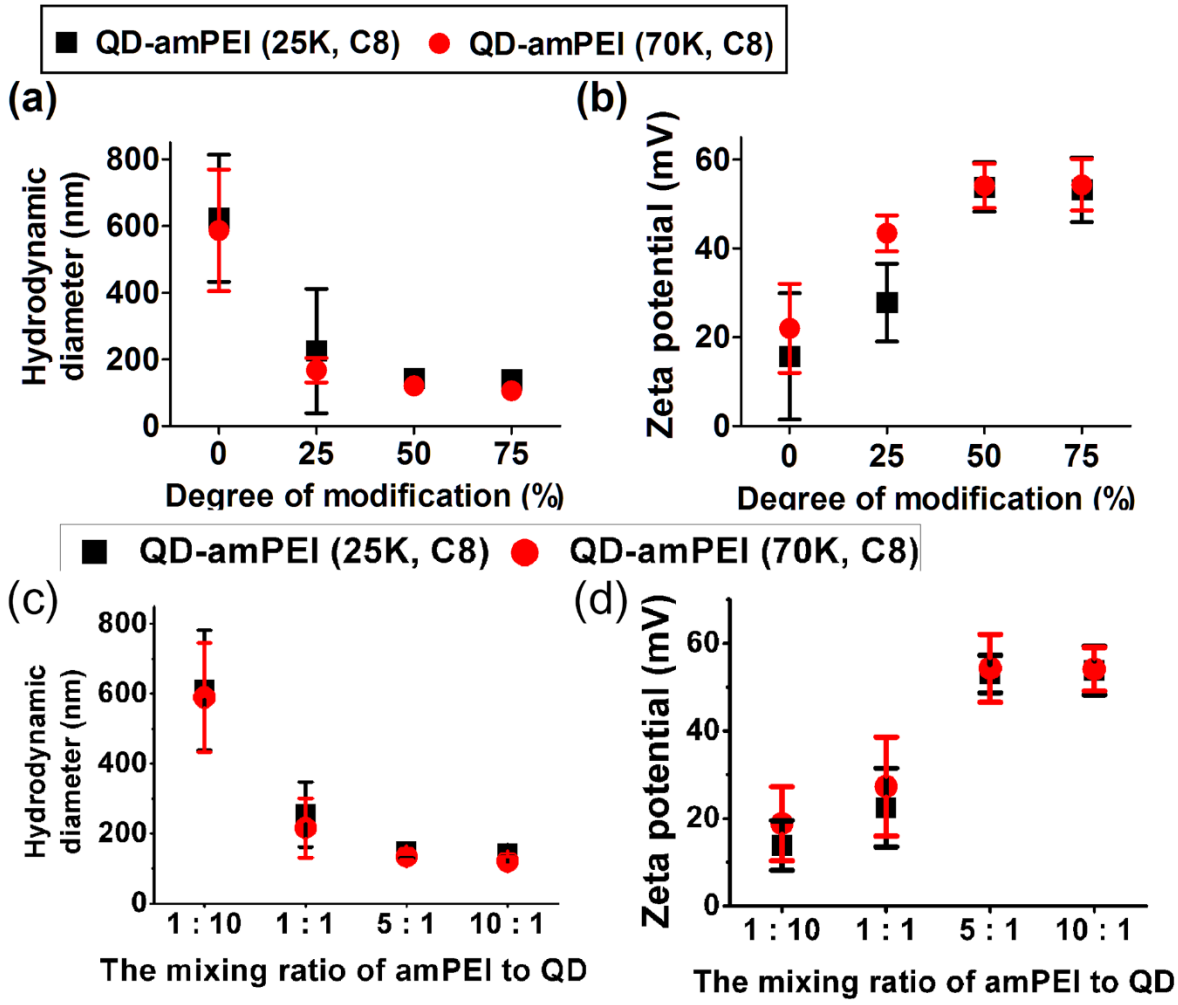

Figure S5. Hydrodynamic diameter and zeta potential values for unmodified polyethyleneimine (PEI) capped QDs (designated with 0 degree of modification in a and b) and amphiphilic PEI polymer (octylisocyanate derivatized PEIs, degree of modification: 25, 50, and 75%) with two different molecular weight (25K and 70K) wrapped QDs. (a) Hydrodynamic diameter and (b) zeta potential values of QD-amPEI(25K, C8) (black squares), and QD-amPEI(70K, C8) (red circles) with various degree of modifications from 0 to 75%. (c) Hydrodynamic diameter and (d) zeta potential values of QD-amPEI(25K, C8) (black squares) and 7 QD-amPEI(70K, C8) (red circles) with different mixing ratio of amPEI to QD from 1/10 to 10 (with 50% degree of modification).

QD samples	Intensity mean value of hydrodynamic diameter (standard deviation) (nm)	Zeta potential (standard deviation) (mV)
QDs (hydrophobic surface)	7.60 (0.43)	N.A.
QD-amPEI(70K, C5)	153 (8.41)	52.2 (2.83)
QD-amPEI(70K, C8)	121 (12.5)	54.1 (4.97)
QD-amPEI(70K, C16)	105 (19.3)	61.5 (5.08)
QD-amPEI(25K, C16)	143 (9.27)	45.0 (2.65)
QD-amPEI(750K, C16)	104 (14.0)	65.1 (1.04)
QD-PEI(25K)	423 (211)	31.7 (0.928)
QD-PEI(70K)	178 (17.5)	53.3 (3.78)
QD-PEI(750K)	138 (10.1)	43.1 (1.84)
CTAB-QDs	72.1 (16.2)	22.3 (2.21)
Lipo-QDs	70.3 (18.1)	55.3 (1.53)
(+)-QDs	11.2 (0.71)	39.6 (1.35)
(-)-QDs	13.7 (2.83)	-18.3 (1.58)
Lipo-(-)-QDs	89.3 (22.1)	50.2 (2.17)

Table S1. Intensity mean value of hydrodynamic diameter and zeta potential values for QD-amPEI composites and their control QD samples. (N. A.: non available)

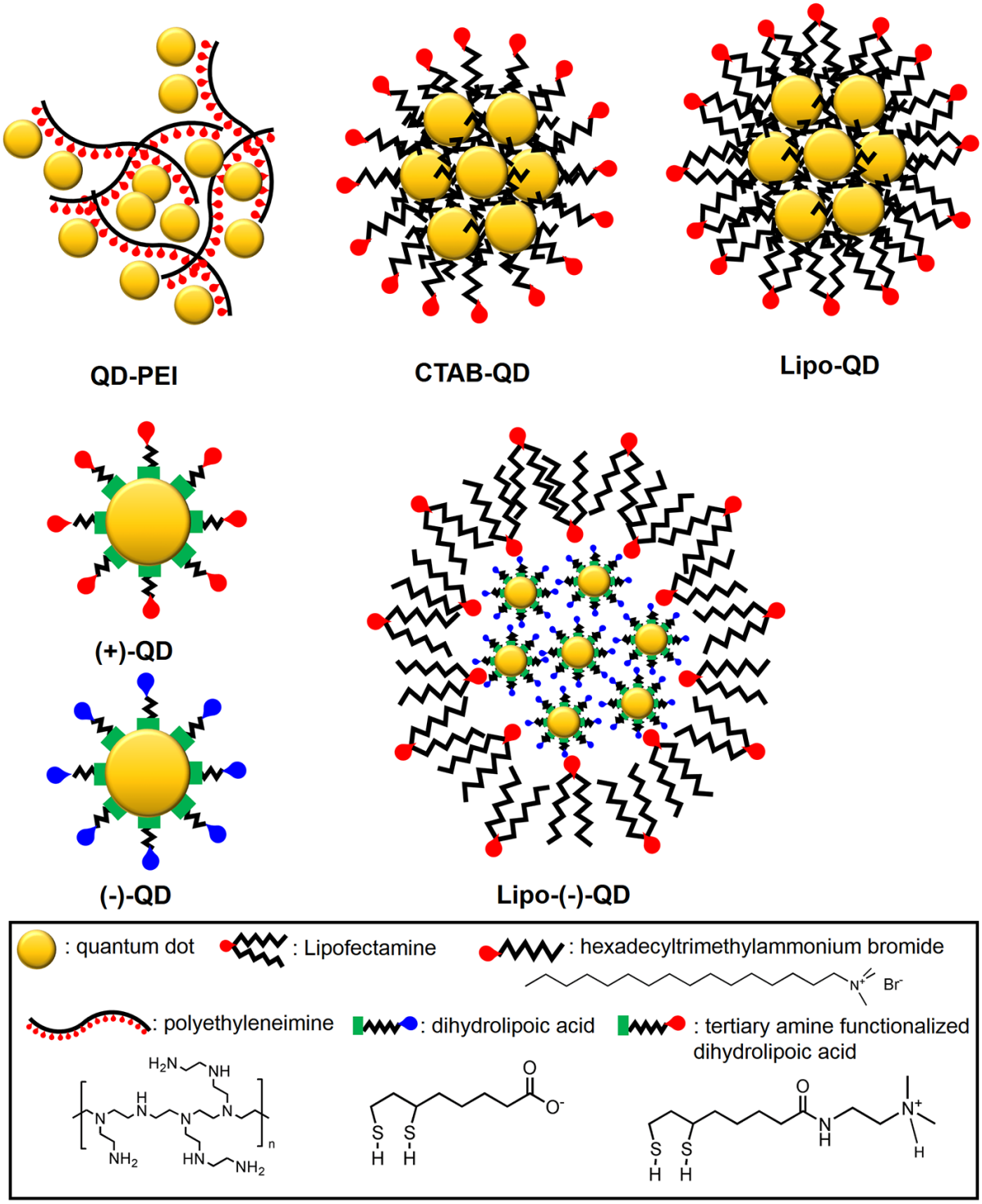

Figure S6. Schematic representations for QD-PEI, CTAB-QD, Lipo-QD, (+)-QD, (-)-QD, and Lipo-(-)-QD

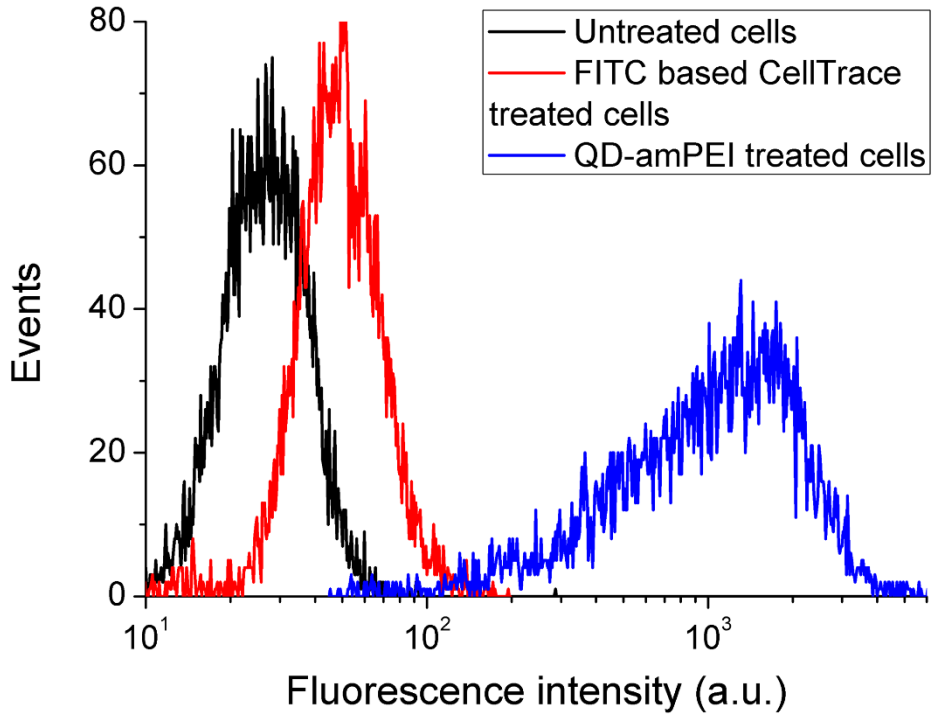

Figure S7. Flow cytometry histogram plots for the fluorescence intensities of untreated HeLa cells (black), CellTrace treated cells (red), and QD-amPEI(70K, C16) treated cells (blue).

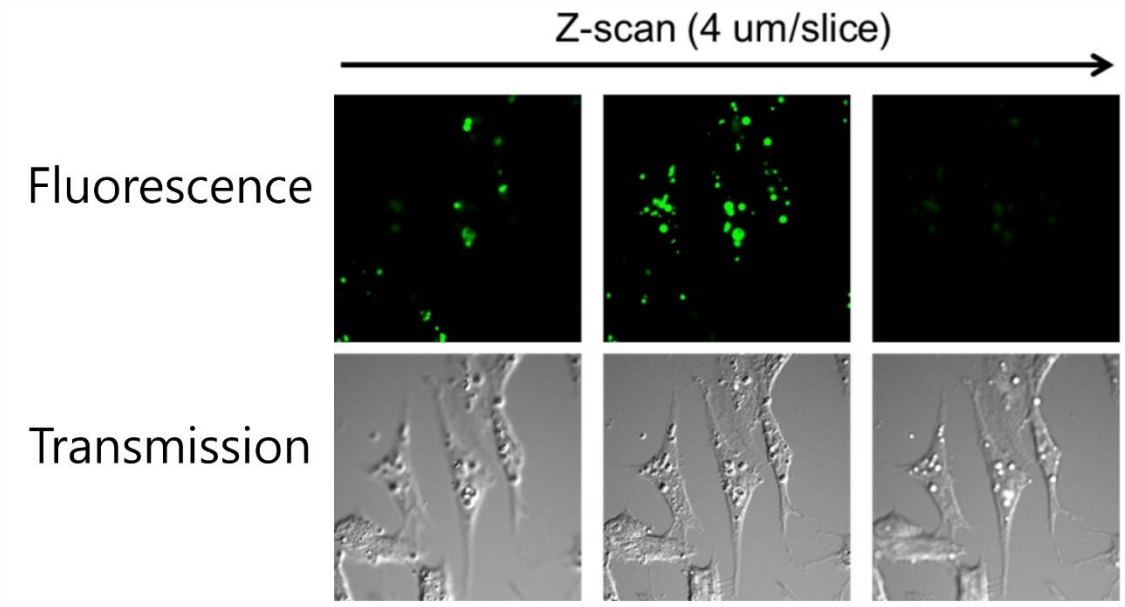

Figure S8. Confocal images of z-axis sectioned HeLa cells labeled with QD-amPEI(70K, C16).

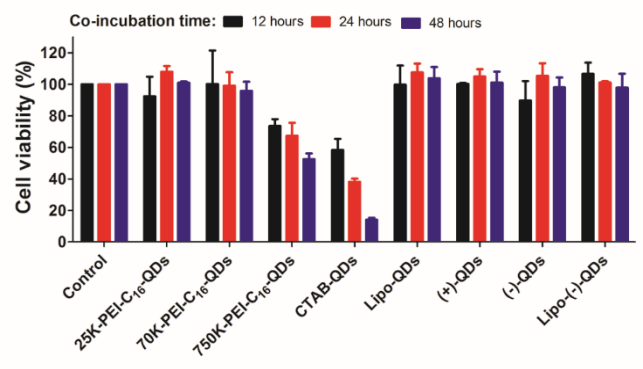

Figure S9. Bar graphs showing the cytotoxicity of HeLa cells labeled with 10 nM QD samples (QD-amPEI (25K, C16), QD-amPEI (70K, C16), QD-amPEI (750K, C16), CTAB-QD, Lipo-QD, (+)-QD, (-)-QD, and Lipo-(-)-QD with different co-incubation time (black: 12 hours, red: 24 hours, blue: 48 hours).

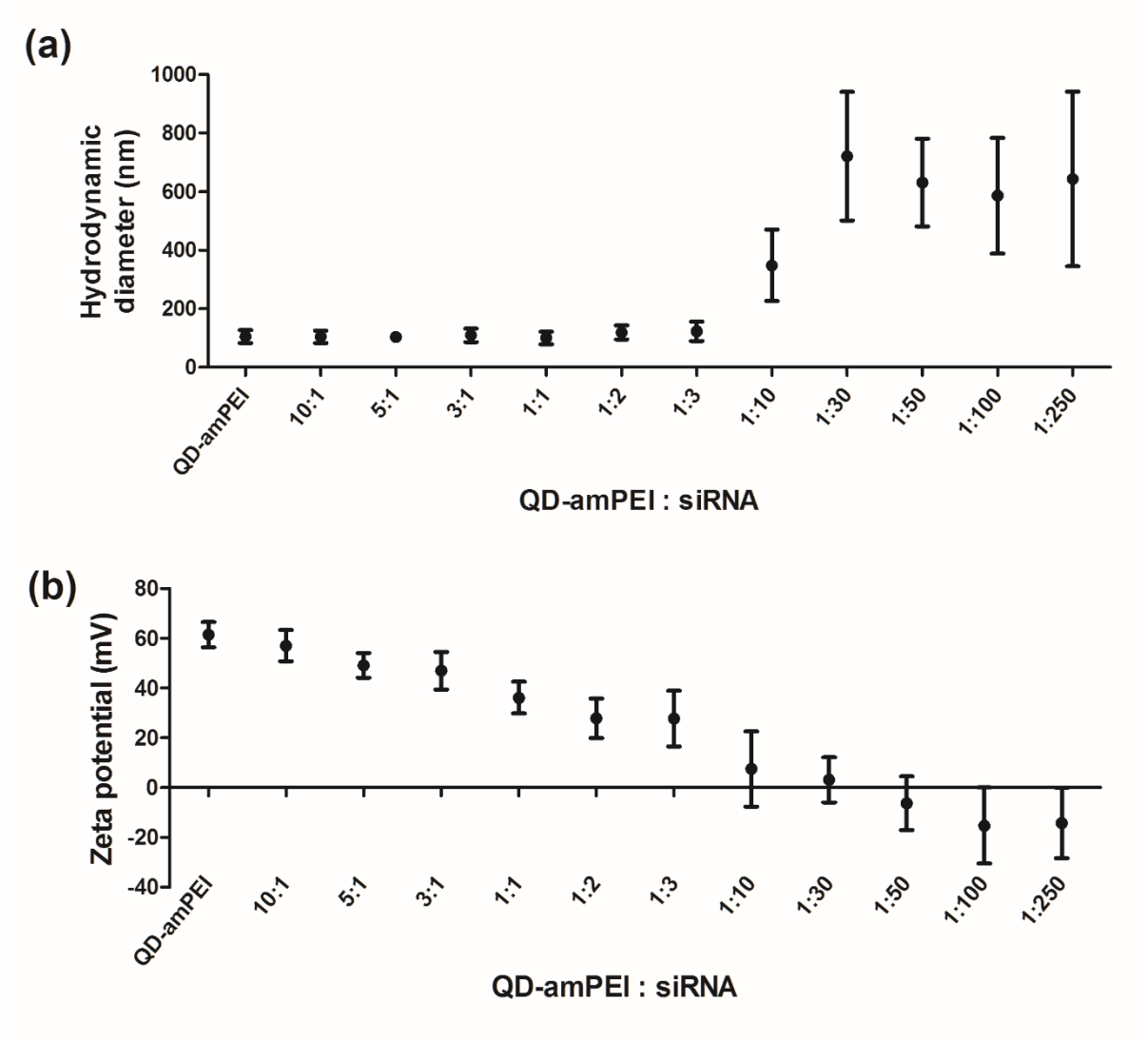

Figure S10. Graph showing (a) hydrodynamic diameter and (b) zeta potential values of QD-amPEI or QD-amPEI-siRNA composites with mixing ratio of amPEI and siRNA with from 10:1 to 1:250.

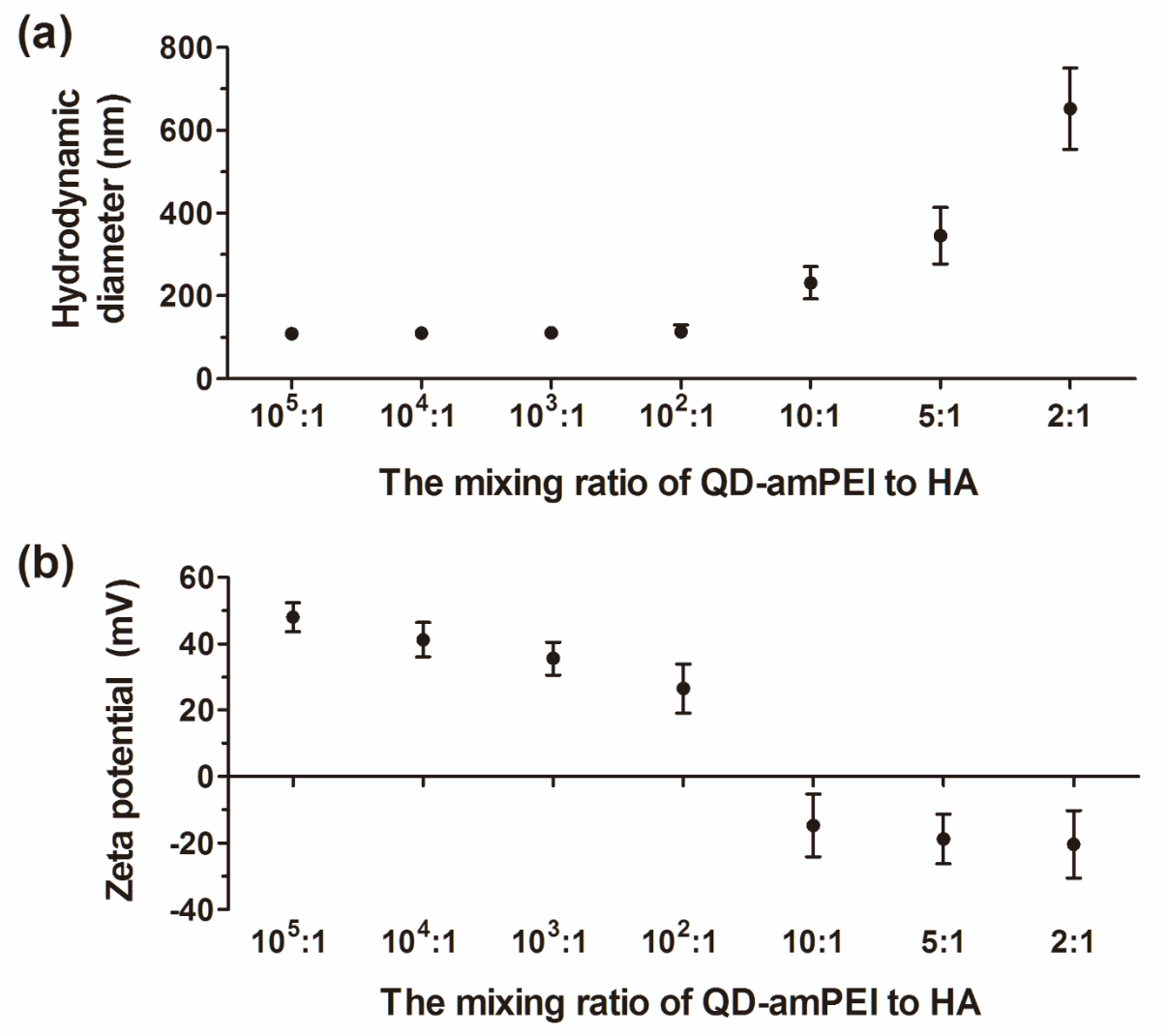

Figure S11. Graph showing (a) hydrodynamic diameter and (b) zeta potential values of HA-QD-amPEI composites with various mixing ratio of QD-amPEI to hyaluronic acid(HA) from 10^5:1 to 2:1.

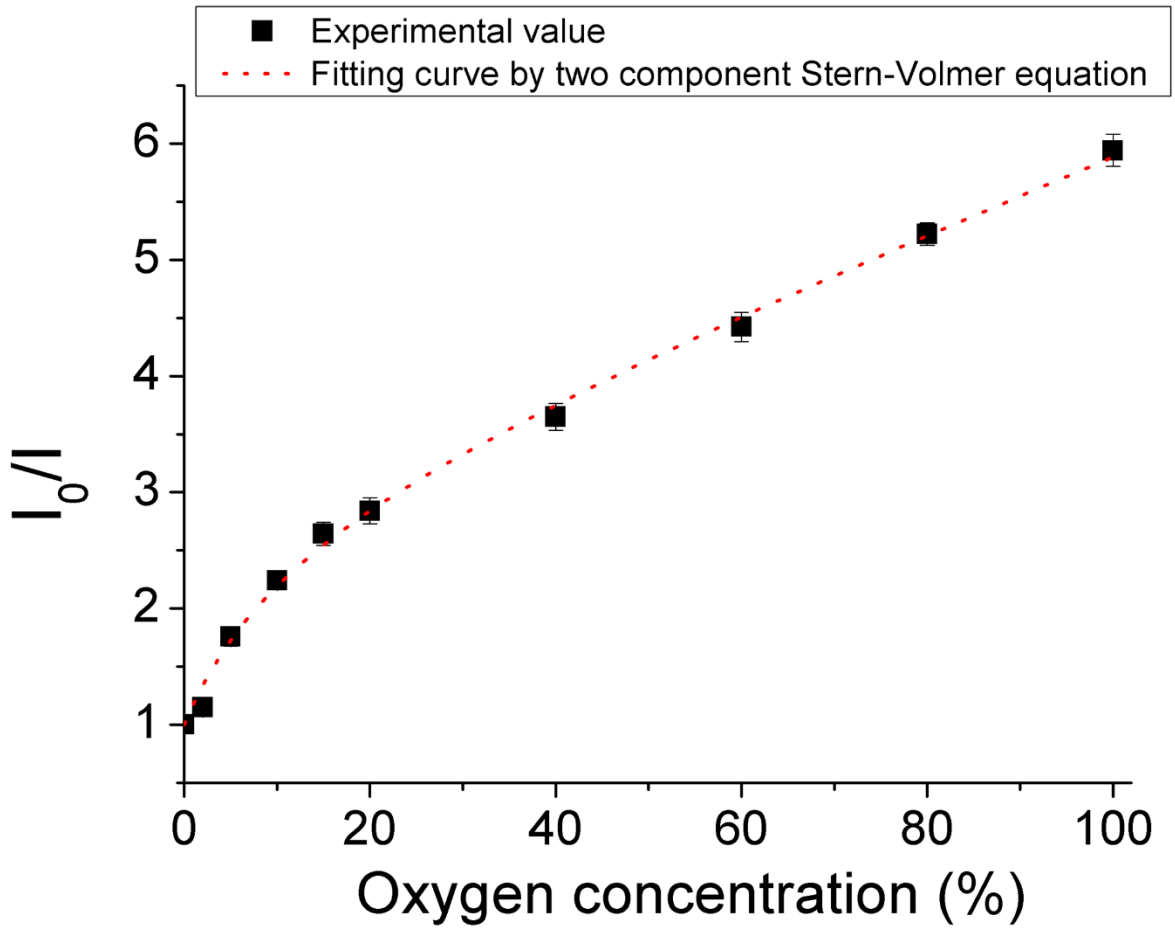

Figure S12. Oxygen sensitive calibration plots of phosphorescence intensity ratio (I_o/I, I_o: phosphorescence intensity at 0% oxygen concentration, I: phosphorescence intensity at ambient condition (0, 2, 5, 10, 15, 20, 40, 60, 80, and 100%) of $Ru(dpp)_3^{2+}$ in QD-Ru-amPEI oxygen probes vs. oxygen concentration was shown.

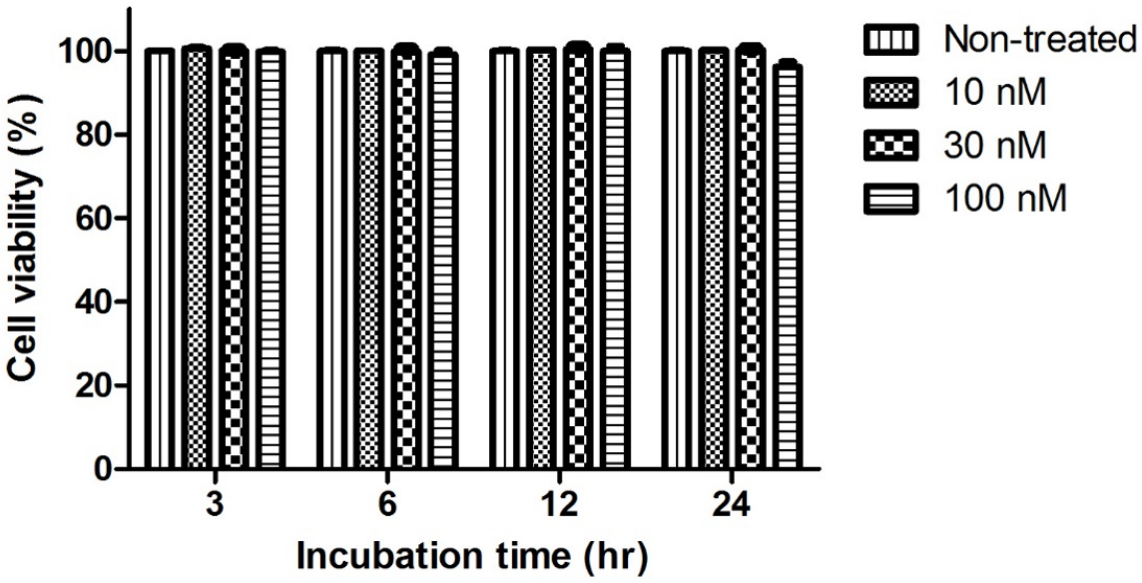

Figure S13. Bar graph showing cell viabilities of HCT116 cells treated by 10, 30, 100 nM QD-Ru-amPEI oxygen probes for 3, 6, 12, and 24 hours.

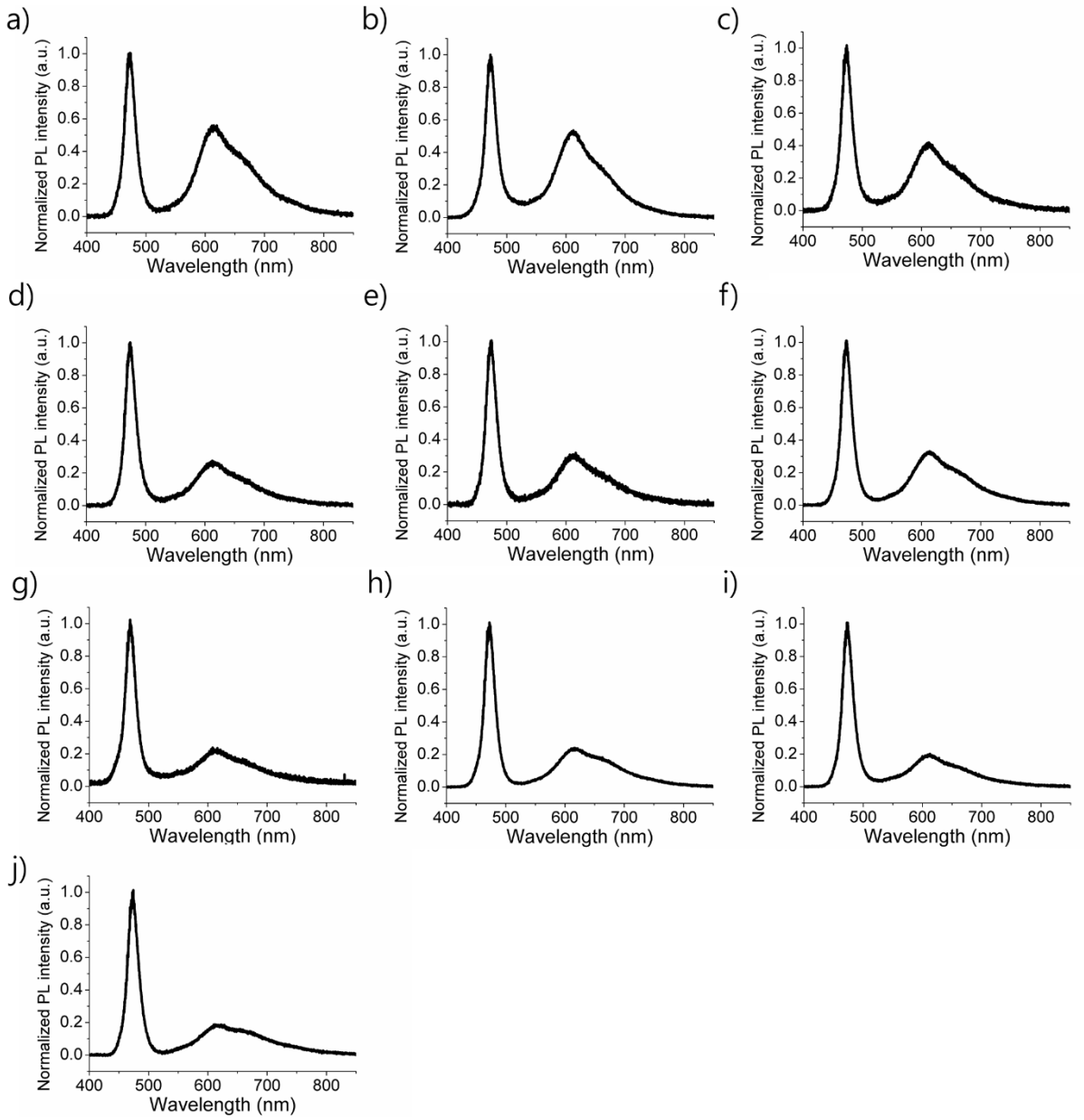

Figure S14. Graph showing normalized PL intensity of spheroid HCT116 cell labeled with QD-Ru-amPEI composites in figure 3d in different regions of interest, (a~c) a1~a3, (d~f) b1~b3, (g~j) c1~c4, respectively.

Reference

1. Chen, D.; Zhao, F.; Qi, H.; Rutherford, M.; Peng, X. Bright and Stable Purple/Blue Emitting CdS/ZnS Core/Shell Nanocrystals Grown by Thermal Cycling Using a Single-Source Precursor. *Chem. Mater.* **2010,** *22*, 1437-1444.