Supporting Information

Imaging of Brain Tissue Using CdSe/CdS Core/Shell Quantum Dots for Correlative Fluorescence and Electron Microscopy

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1. Chemicals

Cadmium oxide (≥ 99.99%), oleic acid (technical grade, 90%), 1-Octadecene (technical grade, 90%), oleylamine (technical grade, 70%), tri-n-octylphosphine (TOP, technical grade, 90%), tri-n-octylphosphine oxide (TOPO, 99%), 1-Octanethiol (98+%), 11-mercaptoundecanoic acid, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. All chemicals were used without further modification.

2. Instrumentation

Absorption spectra were measured using an Agilent Technologies Cary-60 UV-vis spectrometer. Photoluminescence spectra were measured using a Horiba FluoroMax Plus fluorometer. To measure the photoluminescence quantum yield (QY), the Horiba FluoroMax Plus fluorometer was equipped with a 152 mm diameter integrating sphere (Quanta-Phi, Horiba Scientific). The corresponding solvent for each QD sample, PBS/BSA solution and hexane, was used as a blank measurement. The QY of the QDs was measured at an excitation wavelength of 400 nm at an optical density of ≤0.05 at the excitation wavelength to minimize the inner filter effect. All measurements were carried out using a standard 1 cm quartz fluorescence cuvette. Time-resolved photoluminescence decay was measured using a time-correlated single-photon counting (TCSPC, PicoHarp) with pulsed laser excitation at 405 nm and the emission filter through a 620 ± 40 nm bandpass filter. Tissue sections were imaged on a Nikon Ti2-E microscope equipped with a Prime 95B sCMOS camera (Teledyne Photometrics, Tucson, AZ) for fluorescence microscopy, and JEOL 1,400 transmission electron microscope with an EMT Nanosprint-43 Mark II digital camera (Advanced Microscopy Techniques, Woburn, MA).

3. Synthesis of CdSe/CdS core/shell QDs

CdSe/CdS QDs were synthesized according to previous literature with minor modification [1]. To prepare a 0.2 M cadmium oleate precursor, 0.2568 g of cadmium oxide, 3.8 ml of oleic acid, and 6.2 ml of 1-octadecene were added to a three-neck round-bottom flask and placed under vacuum at 100 °C for 1 hour. The mixture was then heated up to 180 °C under nitrogen for 10 minutes until a clear solution was obtained.

To synthesize the CdSe core, 0.1 ml of the previously prepared cadmium oleate, 0.1 ml of oleylamine, 0.3 g of TOPO, and 4 ml of 1-octadecene were added to a three-neck round-bottom flask and placed under vacuum at 100 °C for 1 hour. The mixture was then heated up to 240 °C under nitrogen and the selenium precursor (16 mg of selenium powder dissolved in 0.7 ml 1-octadecene) was injected quickly into the flask and allowed to react for 1 minute. The reaction mixture was then quenched by removing it from the heating mantle. The CdSe core was then washed with ethanol, centrifuged at 7500 rpm for 10 minutes and redispersed in hexane.

To synthesize the CdSe/CdS core /shell QDs, 10 nmol of CdSe core solution, 4 ml of oleylamine and 4 ml of 1-Octadecene were added to a three-neck round-bottom flask and placed under vacuum at 100 °C for 1 hour. The reaction mixture was then heated to 290 °C under nitrogen. Once the reaction mixture reached 240 °C , a syringe pump was used to deliver a desired amount of cadmium precursor (0.2 M cadmium oleate diluted to 3 ml in 1-Octadecene) and sulfur precursor (1.2 equivalent amount of 0.2 M octanethiol diluted to 3 ml in 1-Octadecene) at a rate of 1.5ml/h. After infusion, the reaction mixture was removed from the heating mantle, and 1 ml of oleic acid was added. The reaction mixture was then washed with ethanol, centrifuged at 7500 rpm for 10 minutes, and redispersed in hexane.

4. QD surface functionalization

Ligand exchange was performed to transfer the QDs from the organic phase to an aqueous phase using 11-mercaptoundecanoic acid (MUA). 1 ml of the synthesized CdSe/CdS QDs in hexane were transferred to a vial, dried under a stream of nitrogen gas, and then redispersed in 10 ml of chloroform. Then, 0.1 g of MUA was added, and the solution was sonicated until the MUA was fully dissolved. 5 ml of 0.09 M KOH solution was added to the QD-chloroform mixture, which was then shaken vigorously to form a milky orange solution. The mixture was centrifuged

at 6000 rpm for 1 minute, and the resulting aqueous layer was separated and washed with methanol at a 3:1 ratio. This was followed by another centrifugation at 6000 rpm for 2 minutes. Finally, the precipitate was redispersed in 1 mL of water.

For conjugation of the QDs to streptavidin, 1ml of QD-MUA at a concentration of 0.5 μM was reacted with 1 mM of EDC and NHS for 20 minutes. The mixture was then centrifuged with a 10,000 MWCO filter and redispersed in 1 ml of 1% PBS/BSA. 30 μl of 2 μM of streptavidin was added and allowed to react for 2 hours. The reaction mixture was then centrifuged with a 100 kDa MWCO filter to remove excess unbound streptavidin and redispersed in 1% PBS/BSA. This procedure was repeated three times.

5. Tissue collection and embedding

Male rats were deeply anesthetized with chloral hydrate (750 mg/kg), then transcardially perfused with 500 ml of 4% freshly depolymerized paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. Brains were immersed in the perfusion fixative for 1 hour at room temperature prior to rinses in perfusion buffer. Sections (100 μm) were collected in a vibratome (Leica Biosystems, Wetzlar, Germany), and the region of interest was isolated with a 2 mm biopsy punch.

To prepare tissue for electron microscopy, brain punches were frozen in a Wohlwend Compact 3 high-pressure freezer (Technotrade International, Manchester, NH) and stored under liquid nitrogen until further processing. Frozen samples were processed for embedding in an automated freeze-substitution device (AFS2; Leica Microsystems, Wetzlar, Germany) and freeze-substituted in acetone containing 1% uranyl acetate (Structure Probe, Inc., West Chester, PA) for 72 hours at -90°C. Substituted samples were rinsed in pure acetone before reaching -35°C at a rate of 5°C per hour. Resin infiltration happened at -35°C following incubations with resin diluted in acetone (1:1 and 2:1) and 1mM Dithiothreitol (DTT) for 4 hours each. Pure resin solution with 10mM DTT was incubated overnight, and 1 hour with a fresh resin solution prior to embedding. Tisue samples were transferred into polyethylene flat embedding capsules (Electron Microscopy Sciences) filled with fresh resin and polymerized under a UV-A lamp at -35°C for 48 hours and warmed to 20°C at a rate of 5°C per hour with UV light. Embedded blocks were hand-trimmed with razor blades, and sections were collected (70 nm) on a Leica UC7 ultramicrotome. For immunolabeling, sections were placed on #1.5 glass coverslips and on gold slot grids coated with pioloform (Ted Pella, Inc) for electron microscopy imaging.

6. Tissue immunolabeling

Tissue ultrathin sections (70 nm) were first blocked for 30 minutes in PBS containing 1% bovine serum albumin (BSA; Jackson ImmunoResearch Laboratories Inc. West Grove, PA). Primary antibodies (γ-aminobutyric acid (rabbit anti-GABA at 1:10,000; Sigma Aldrich #A2052, lot#0000375922) diluted in blocking solution was incubated for 1 hour, then rinsed twice for 2 minutes in PBS containing 0.1% Tween 20 (PBST) and followed with an incubation for 30 minutes with secondary biotinylated antibody (goat anti-rabbit biotin conjugate at 1:200; Invitrogen #65-6140). Sections were rinsed twice in PBST prior to a 30-minute incubation with streptavidin-AlexaFluor 647 (1:200; Invitrogen #S32357) or streptavidin-QDs (100 nM) diluted in blocking buffer. Sections were rinsed twice in PBST and mounted in ProlongGold + DAPI (Invitrogen). All incubations were performed at room temperature in a humidified plastic tray to prevent section drying.

7. Tissue immunolabeling for electron microscopy

Tissue ultrathin sections (70 nm) were collected on gold pioloform coated grids (Ted Pella, Inc.). All immunolabeling steps were performed by placing drops of buffer solution on top of parafilm placed in a humidified plastic tray. Grids were placed on top of the drops of buffer with tissue facing down. First, grids were rinsed once for 5 minutes in tris buffer (TBS, 0.1M Tris-HCl, 137mM NaCl, 2.7mM KCl, pH 7.4), then blocked for 30 minutes with 1%BSA-c (Aurion, Netherlands), 1% cold water fish skin gelatin (Electron Microscopy Sciences), 35mM lysine and 1% normal goat serum (ThermoFisher #31873) in TBS pH 7.4. Primary antibody (rabbit anti-GABA at 1:1000; Sigma

Aldrich #A2052, lot#0000375922) diluted in blocking buffer was incubated for 1 hour, then grids were rinsed in TBS and incubated with biotinylated antibody (goat anti-rabbit biotin conjugate at 1:200; Invitrogen #65-6140) for 30 minutes. After rinsing in TBS, grids were incubated with streptavidin-QDs at 200 nM in blocking buffer for 30 minutes and rinsed in TBS prior to a last rinse in distilled water for 5 minutes. All TBS rinses were done three times for 5 minutes each. Grids were allowed to dry at room temperature and stained with UA and lead. Staining was done by placing grids with tissue facing down on drops of 2% uranyl acetate in water for eight minutes and three minutes in lead citrate. Rinses are performed by dipping the grids in water five times for 20 seconds each. The grids are left to fully dry for 20 minutes between each staining step and before imaging.

8. Additional Experimental Results

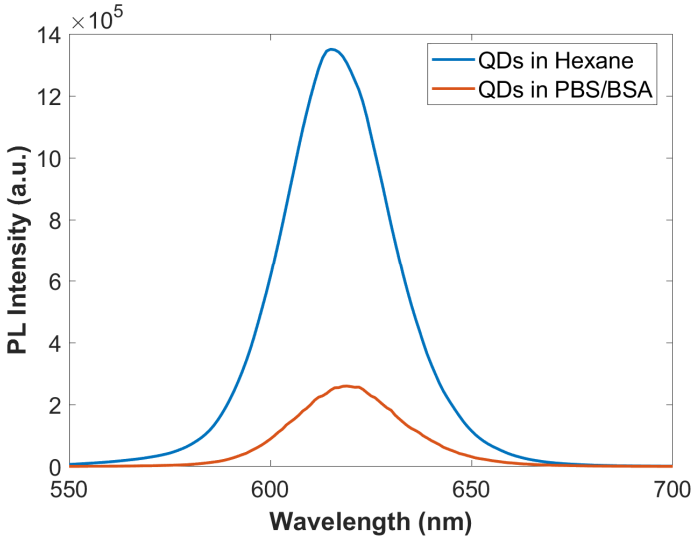

Figure S1. Photoluminescence spectra of CdSe/CdS QDs before and after MUA ligand exchange. The as-synthesized QDs were measured in hexane, while the ligand-exchanged QDs were measured in PBS/BSA at the same concentration (200 nM). Both samples were excited at 400 nm.

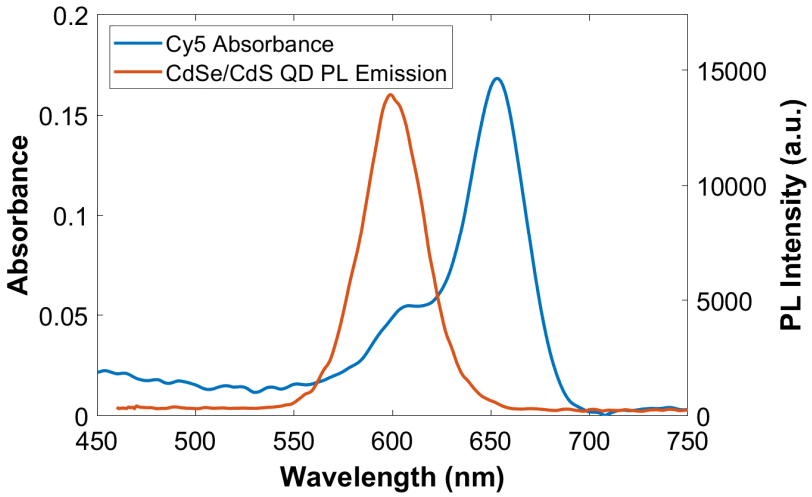

Figure S2. Spectral overlap between CdSe/CdS QD emission and Cy5 absorption, enabling FRET between the QD donor and Cy5 acceptor.

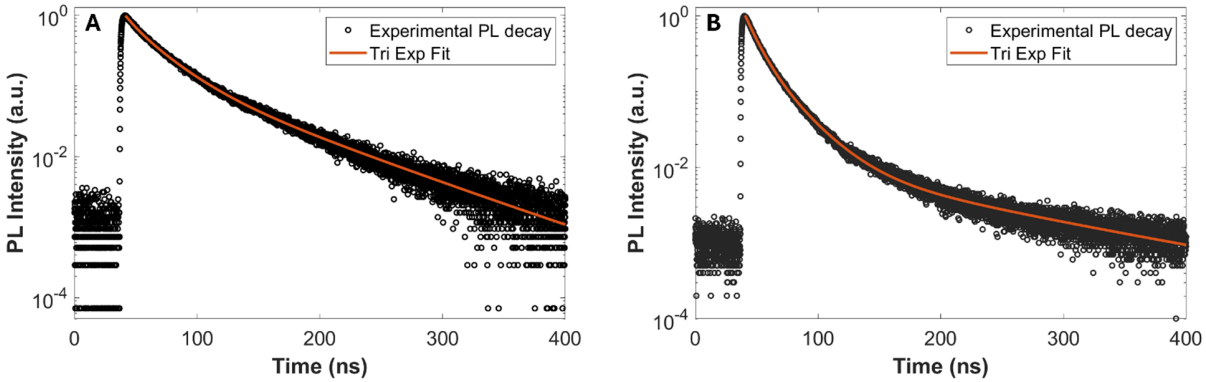

Figure S3. Experimental PL decay curves and tri-exponential fitting for streptavidin-functionalized QDs in the (**A**) absence and (**B**) conjugated to Cy5 Biotin.

Table S1. Fitting parameters A_i, τ_i, and τ_{avg} for QD-Streptavidin in the absence and conjugated to Cy5 Biotin. The PL decays were fit to a tri-exponential function: $I(t) = \sum_i A_i e^{-t/\tau_i}$, and the weighted average lifetime was determined by: $\tau_{avg} = \sum_i A_i \tau_i^2 \Big/ \sum_i A_i \tau_i$.

	A_1	τ_1 (ns)	A_2	τ_2 (ns)	A_3	τ_3 (ns)	τ_{avg}(ns)
QD-Streptavidin	21.17	8.72	3.49	24.55	0.31	17.90	12.55
QD-Streptavidin + Cy5 Biotin	46.38	9.58	1.85	23.74	0.02	131.25	11.42

Reference

1. Wang, X.; Chen, S.; Thota, S.; Wang, Y.; Tan, H.; Tang, M.; Quan, Z.; Zhao, J. Anisotropic Arm Growth in Unconventional Semiconductor CdSe/CdS Nanotetrapod Synthesis Using Core/Shell CdSe/CdS as Seeds. *J. Phys. Chem. C* **2019**, *123*, 19238–19245.