Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Ligand-Conjugated Quantum Dots for Fast Sub-Diffraction Protein Tracking in Acute Brain Slices

Lucas B. Thal[a,b,c], Victor R. Mann[d,e], David Sprinzen[f,g], James R. McBride[a,c], Kemar R. Reid[a,c], Ian D. Tomlinson[a], Douglas G. McMahon[g], Bruce E. Cohen*[eh], and Sandra J. Rosenthal*[a,b,c,i,j,k]

[a]Department of Chemistry, Vanderbilt University, Nashville, TN 37235

[b]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37235

[c]Vanderbilt Institute of Nanoscale Science and Engineering, Vanderbilt University, Nashville, TN 37235

[d]Department of Chemistry, University of California, Berkeley, California 94720

[e]The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720

[f]Neuroscience Graduate Program, Vanderbilt University, Nashville, TN 37235

[g]Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235

[h]Molecular Biophysics and Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720

[i]Department of Pharmacology, Vanderbilt University, Nashville, TN 37235

[j]Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN 37235

[k]Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37235

* Co-corresponding authors

Methods

Synthesis of symmetrically shelled CdSe/CdS QDs. CdSe cores were synthesized as previously described.[1-3] In an Ar glovebox, 0.058 g of Se powder (Aldrich, 99.99% trace metal basis) was added to 0.36 g of trioctylphosphine (TOP, Aldrich, 97%) in a glass vial and stirred overnight, yielding TOP-Se as a clear, colourless solution. On a Schlenk line, 0.060 g of CdO (Aldrich, 99.99% trace metal basis, 0.47 mmol), 0.28 g of octadecylphosphonic acid (ODPA, 0.84 mmol), and 3.0 g of TOP oxide were combined in a 50-mL flask fitted with a condenser and temperature probe. The flask was heated under N_2 to 150 °C and stirred under vacuum for 1 hr. The flask was purged with N_2 and heated to 320 °C until the Cd complexed with the ODPA to become clear and colourless. TOP (1.50 g) was injected into the flask drop-wise and the temperature was then raised to 370 °C. The TOP-Se precursor was then rapidly injected, and allowed to react for 70 sec. The flask was cooled with air to below 110 °C, and 2 mL of dry ice-cooled toluene was injected. The final reaction solution was removed and the total volume of the solution was diluted with toluene to 15 mL. The particles were then precipitated with 15 mL of acetone and centrifuged at 4000 x *g* for 5 min. The pellet was dispersed in a minimum of $CHCl_3$, precipitated with 10 mL of acetone, centrifuged at 4000 x *g* for 5 min, dispersed in a minimum of hexane (~1 mL), and precipitated with acetone again. These cores were dispersed in 5 mL of hexane and stored in a glovebox.

Stock solutions of 0.1 M Cd oleate in 1-octadecene (ODE) and 0.1 M octanethiol in ODE were prepared in a glovebox. On a Schlenk line, 5 mL of ODE was placed in a 3-neck flask under N_2 and 100 nmol of CdSe core nanocrystals in hexane were injected. Solvent was removed under vacuum at room temperature and then at 120 °C for 20 min. The reaction was purged with N_2 and the glovebox solutions containing 7 mL of 0.1 M Cd oleate in ODE and 7 mL of 0.1 M octanethiol in ODE were injected at 310 °C *via* syringe pumps over 2.5 hr. After injection, 1 mL of oleic acid (OA) was quickly injected and the reaction maintained at 310 °C for 1 hr. The reaction flask was cooled with air, the nanocrystals cleaned by repeated precipitation as above, and the nanocrystals dispersed in 10 mL of hexane with 1% OA (*v/v*) for storage under ambient conditions.

Passivation of core–shell CdSe/CdS QDs by PAOA amphiphilic copolymer. CdSe/CdS QDs with emission maxima of 640 nm were dispersed in hexane with 1% (*v/v*) OA to 3.75 µM, as determined by first exciton absorbance. poly(acrylic acid)-*co*-poly(*n*-octylacrylamide)-*co*-poly(2-aminoethylacrylamide (PAOA, MW ~3000 Da) random copolymer was prepared as previously described.[2, 3] For aqueous dispersion, PAOA (24 mg, 7.5 µmol, 10,000-fold molar excess over QDs) was dissolved in 1 mL of MeOH and 15 mL of $CHCl_3$. QDs in hexane (*e.g.*, 200 µL of 3.75 µM 640 nm CdSe/CdS QDs, 0.75 nmol) were added with stirring, and the solvents were removed under a gentle stream of N_2 overnight. The dried residue was then resuspended in 15 mL of 200 mM sodium bicarbonate buffer, pH 8.0. This suspension was sonicated for 30 min, heated in an 80 °C water bath for 60 min, slowly cooled in the bath to <30 °C, and then sonicated for 30 min. Excess polymer was removed by spin dialysis (Amicon Ultra-15, 50 kDa MWCO), washing with 3 × 15 mL of 100 mM HEPES, pH 7.8. The retentate was diluted to 750 µL with HEPES buffer and centrifuged at 16,100 x *g* for 5 min to remove residual polymer and insoluble aggregates. Aqueous QD dispersions were stored under ambient conditions.

Surface conjugation of ligands. Polymer-encapsulated QDs (0.5 µM, 300 µL) in 0.1 M HEPES, pH 8.0, were mixed with 8.7 µL of 90% 1 mg/mL IDT725-succinimidyl ester (SE) and 10% methoxy-PEG8-SE dissolved in EtOH, and vortexed well. After incubating overnight, the volume was increased to 500 µL with 0.1 M HEPES, pH 8.0, and excess SE reagents were removed via spin dialysis (Amicon Ultra, 30 kDa MWCO) by washing 12 additional times with HEPES.

Dynamic light scattering. Diameters were measured using a Malvern Zetasizer. Aqueous QDs were diluted to ~20 nM with distilled water and filtered through a 0.2-µm cut-off PVDF filter (Pall) before analysis. Typical count rates were 200 kilocounts per second. Data were collected for 150 seconds in

triplicate and fit using Malvern Zetasizer software to a volume-weighted size distribution of hydrodynamic diameter.

Electron Microscopy and Electron Dispersive X-ray Spectroscopy. HRTEM and STEM-EDS were performed on a Tecnai Osiris TEM/STEM operating at 200 kV equipped with a SuperX™ quad EDS detection system. Samples were prepared by drop casting or dip-coating dilute dispersions of the QDs onto ultrathin on lacey carbon support film (TED Pella 1824) and baked at 145 Ċ overnight under high vacuum prior to imaging. STEM-EDS maps were collected using Bruker Esprit software with a sub-nm probe having ~ 0.8 nA of beam current.

Ensemble Spectroscopy. Absorption spectra were collected using a Cary 60 UV-VIS spectrometer. Photoluminescence (PL) spectra were collected using a PTI QuantaMaster fluorescence spectrophotometer equipped with a 75 W Xe arc lamp as the excitation source. PL was acquired in 1 s integration time intervals with a 1 nm slit width.

Time-Resolved Photoluminescence. Time-resolved photoluminescence (TRPL) measurements were performed on dilute solutions of QDs with optical densities below 0.2 at the lowest-energy absorption transition.[4, 5] The QD solutions were excited under wide-field illumination using a 405 nm pulsed source at a 1 MHz repetition rate. PL from the solutions was filtered with an appropriate long-pass filter and directed onto a single-photon avalanche photo-diode (SPAD, Micro Photon Devices, PD-050-0TC). A time-correlated single photon-counting unit (TCSPC, PicoHarp 300) was used to generate a histogram of photon arrival times. Ensemble lifetimes were determined by fitting the histogram of arrival times to a tri-exponential function.

Single QD Fluorescence Analysis. QDs were first diluted to a 100 pM concentration and drop cast on an untreated no. 1.5 MatTek dish. The coverslip was incubated at room temperature for 3 minutes prior to aspiration. The dried coverslips were subsequently treated with either 100 mM HEPES pH 7.8 buffer or oxygenated aCSF. Intensity traces were acquired with a Nikon Eclipse Ti-E inverted microscope equipped with a Yokogawa CSU-X1 spinning-disk head, 1.4 NA 60× oil objective, Andor DU-897 EMCCD.[6] QDs were excited using a dedicated 405 nm laser at 51 W/cm2 with emission collected with a 640 ± 75 nm emission filter. Blinking and photobleaching traces were acquired in 100 ms and 1 s time intervals, respectively. In-house MATLAB routines were used for automated analysis of all imaging data.

Animal handling. All animal care and experiments were conducted in concordance with Vanderbilt University's Institutional Animal Care and Use Committee guidelines. Mice were approximately 1–3 months of age, kept in an environmentally controlled facility (~22 °C, ~40% humidity), caged with no more than five animals from weaning until experimental use on a 12:12 light/dark cycle, and were provided with food and water *ad libitum*.

Acute Brain Slice Preparation and Imaging. Brains were dissected and blocked in cold, oxygenated 95% O_2 aCSF solution (in mM: 114.5 NaCl, 3.5 KCl, 1 NaH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 10 D-glucose and 35.7 $NaCHO_3$). Striatal slices (300 μm) were cut with a Leica vibrotome at 4–10 °C and transferred directly to a continually oxygenated chamber with cold aCSF. Slices were incubated with a mixture of 1X CellMask deep red stain and 50 nM QD-IDT725 for 10 minutes prior to three washes with cold aCSF. QDs in the striatum were visualized using a Nikon Eclipse Ti-E inverted microscope equipped with a Yokogawa CSU-X1 spinning disk head and an Andor DU-897 EMCCD. QDs and the CellMask stain were excited with a 405 nm and 488 nm excitation sources, respectively. Emissions were collected with a 640 ± 75 nm and 700 ± 37 nm emission filters, respectively. In-house MATLAB routines were used for automated analysis of all imaging data. Labelling and tracking protocols were previously detailed.[7]

Figures and Tables

Table S1 Time-resolved PL lifetimes and relative amplitudes of (c) QD655s and (d) symmetrically shelled (symm-shelled) CdSe/CdS QDs.

Sample	τ_1 (ns)	A_1	τ_2 (ns)	A_2	τ_3 (ns)	A_3	τ_{avg}
QD655s in HEPES	3.7 ± 0.3	0.17	21.3 ± 0.5	0.49	47.1 ± 0.5	0.34	27.0 ± 0.2
QD655s in aCSF	4.9 ± 0.4	0.19	19.4 ± 0.5	0.56	42.3 ± 0.6	0.25	22.4 ± 0.1
symm-shelled QDs in HEPES	6.4 ± 0.5	0.15	38.3 ± 0.9	0.59	88.1 ± 1.7	0.26	46.8 ± 0.2
Symm-shelled in aCSF	6.6 ± 0.6	0.13	87.2 ± 1.6	0.28	37.4 ± 0.9	0.59	47.7 ± 0.2

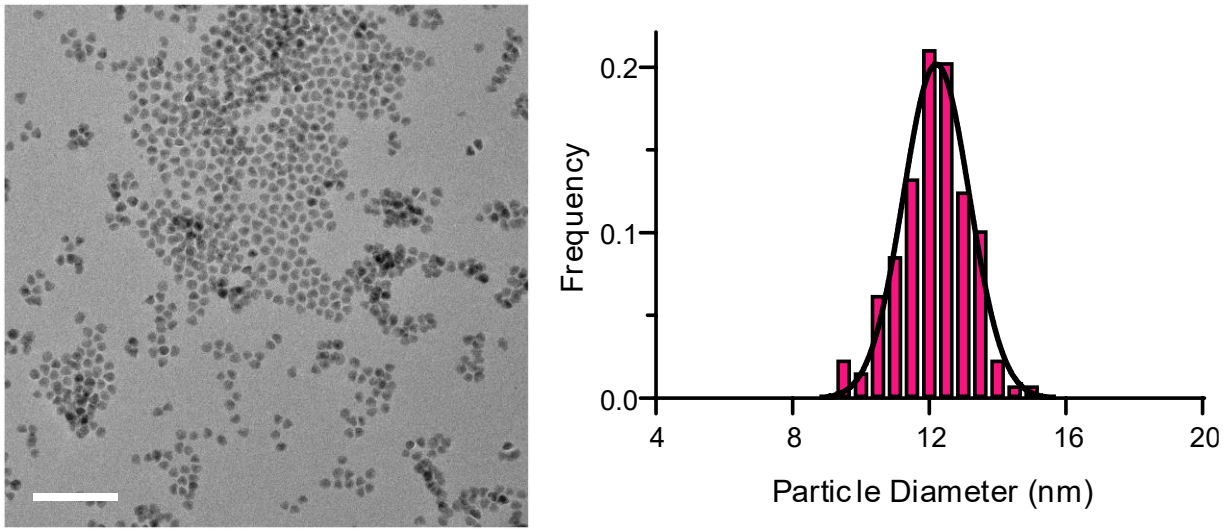

Fig. S1 Transmission electron microscopy (TEM) particle sizing of symmetrically shelled (symm-shelled) QDs.

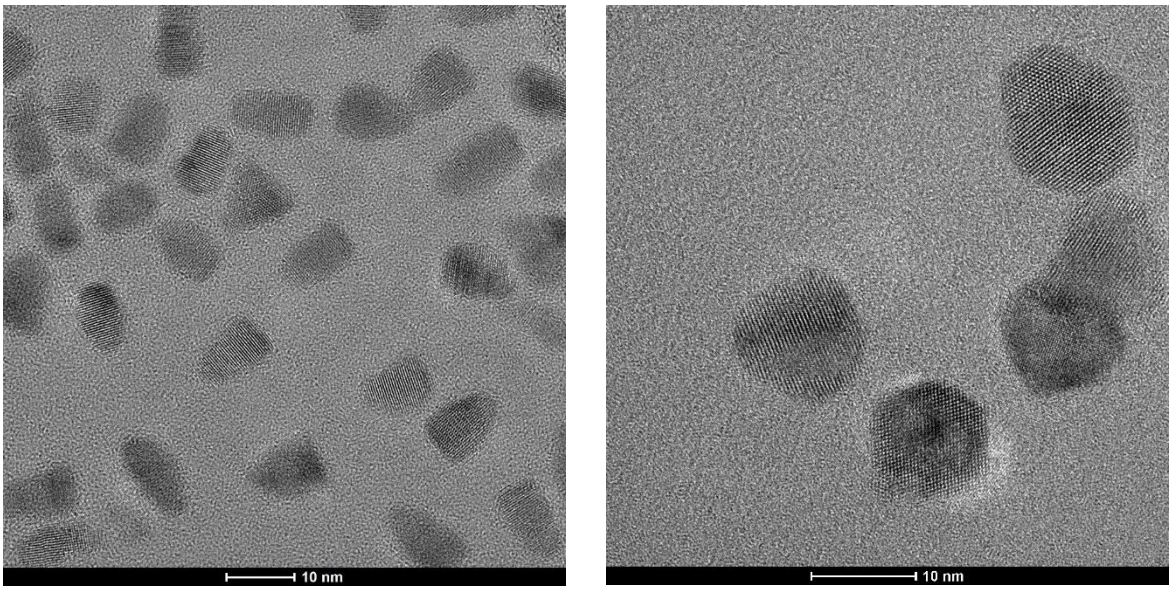

Fig. S2 High-resolution transmission electron microscopy (HRTEM) of (a) QD655s and (b) symm-shelled QDs. Images were acquired at different magnifications.

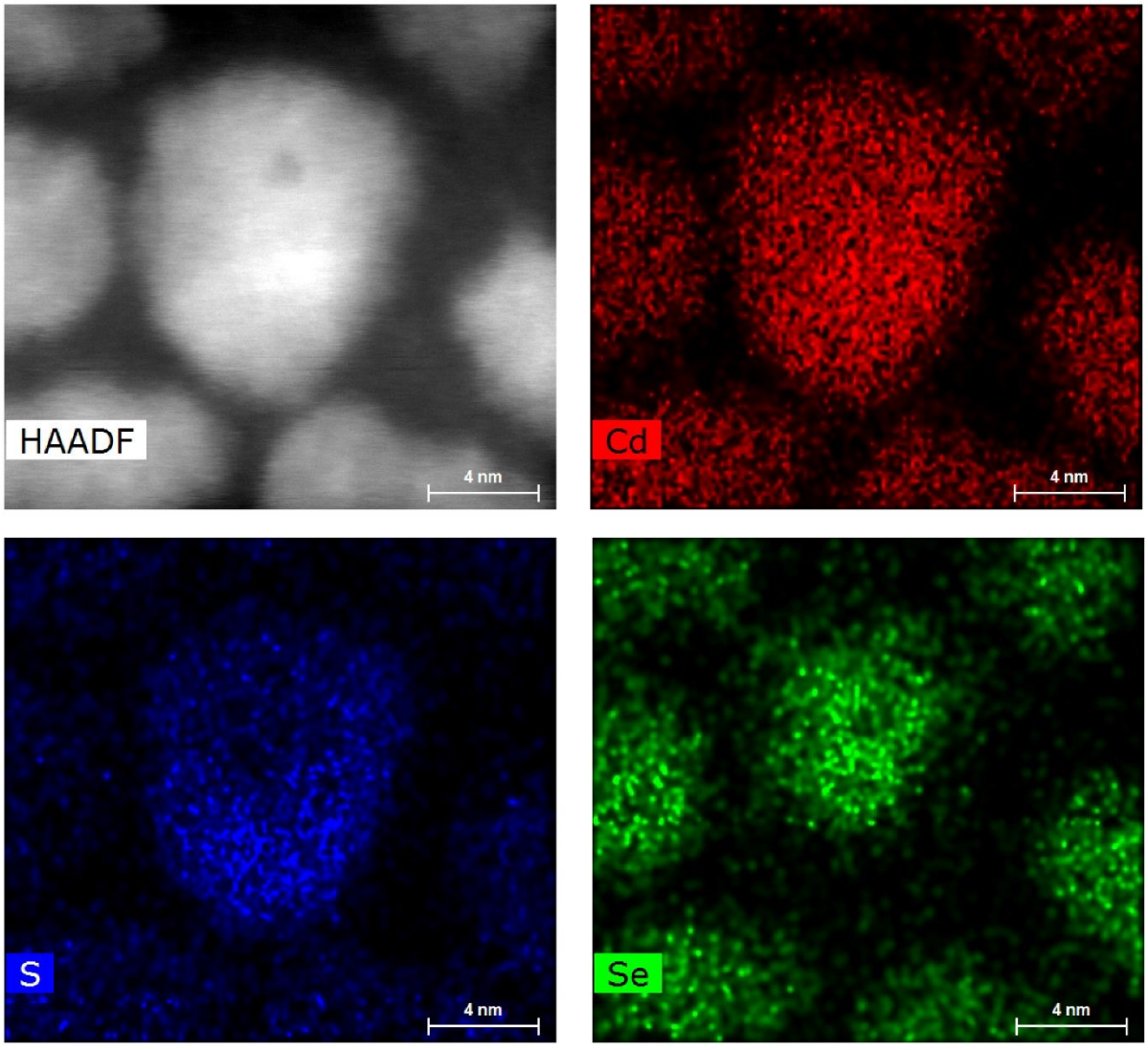

Fig. S3 Individual scanning transmission electron microscopy (STEM) images including high angle annular dark field (HAADF, grey) and Cd (red), S (blue) and Se (green) chemical maps for QD655s.

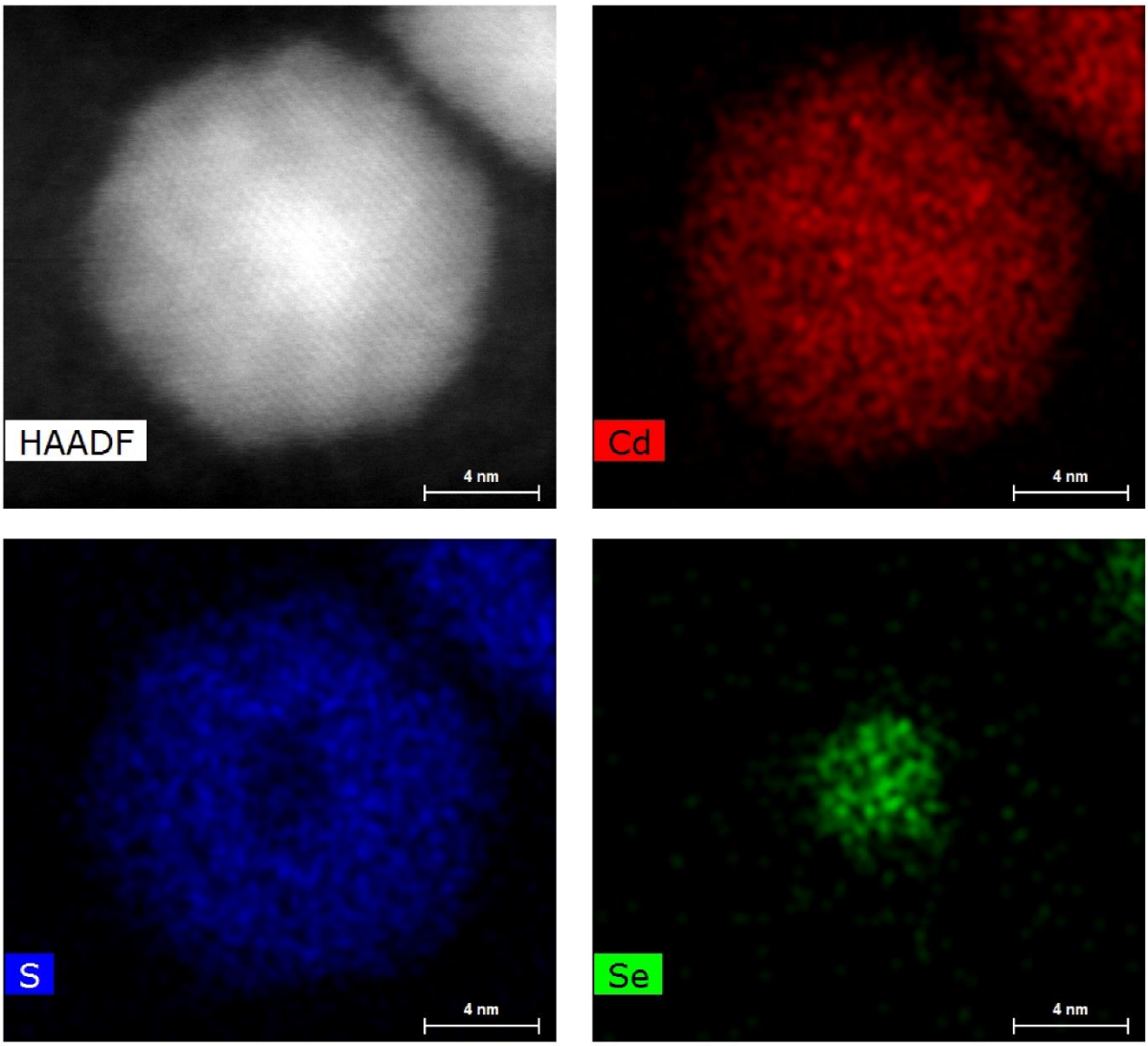

Fig. S4 Individual scanning transmission electron microscopy (STEM) images including high angle annular dark field (HAADF, grey) and Cd (red), S (blue) and Se (green) chemical maps for symmetrically shelled (symm-shelled) CdSe/CdS QDs.

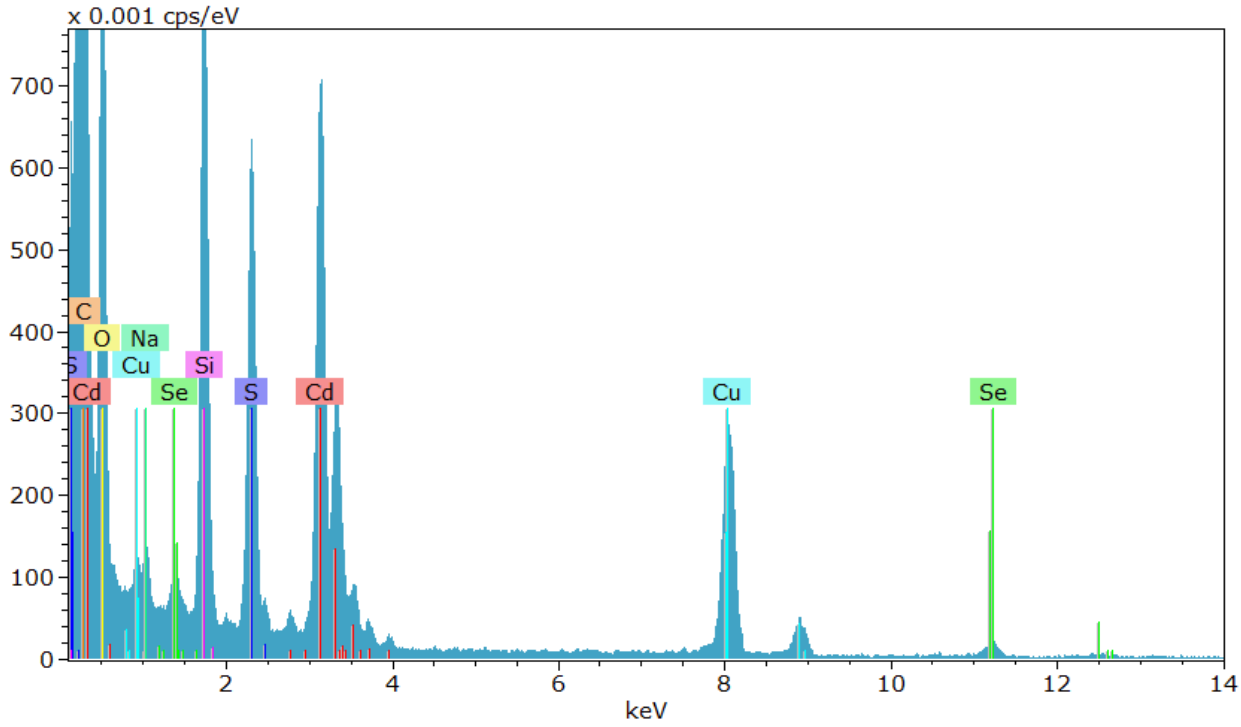

Fig. S5 Electron-dispersive X-ray spectrum of symmetrically shelled (symm-shelled) CdSe/CdS QDs.

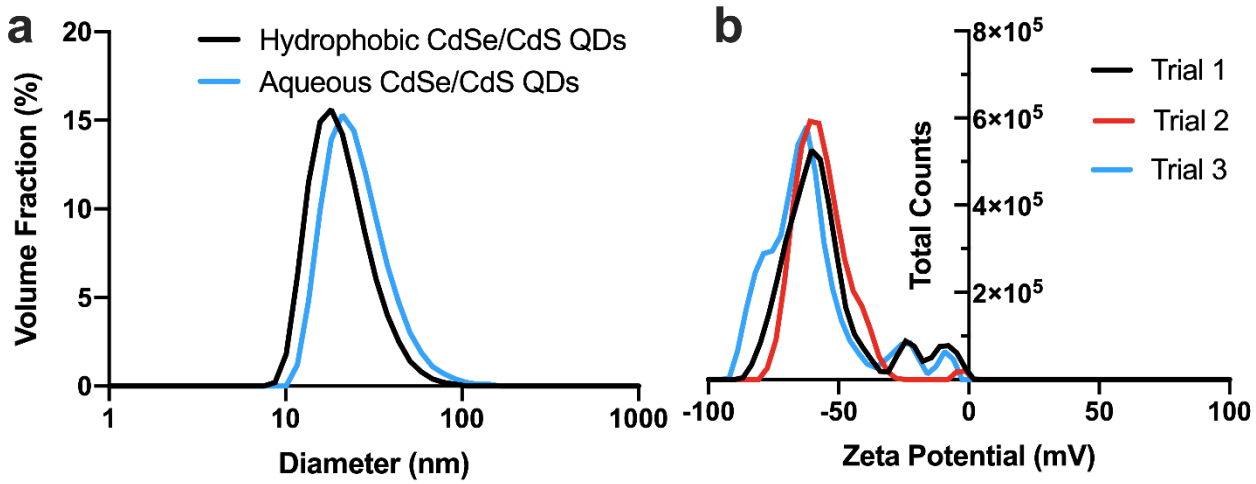

Fig. S6 (a) Dynamic light scattering size measurement of as-synthesized (hydrophobic, black) and PAOA-wrapped (aqueous, blue) symmetrically shelled CdSe/CdS QDs. Peak values found are 18.2 and 21.0 nm, respectively. (b) Three independent zeta potential scans of symmetrically shelled CdSe/CdS QDs with a mean potential of -60.6 ± 9.9 mV.

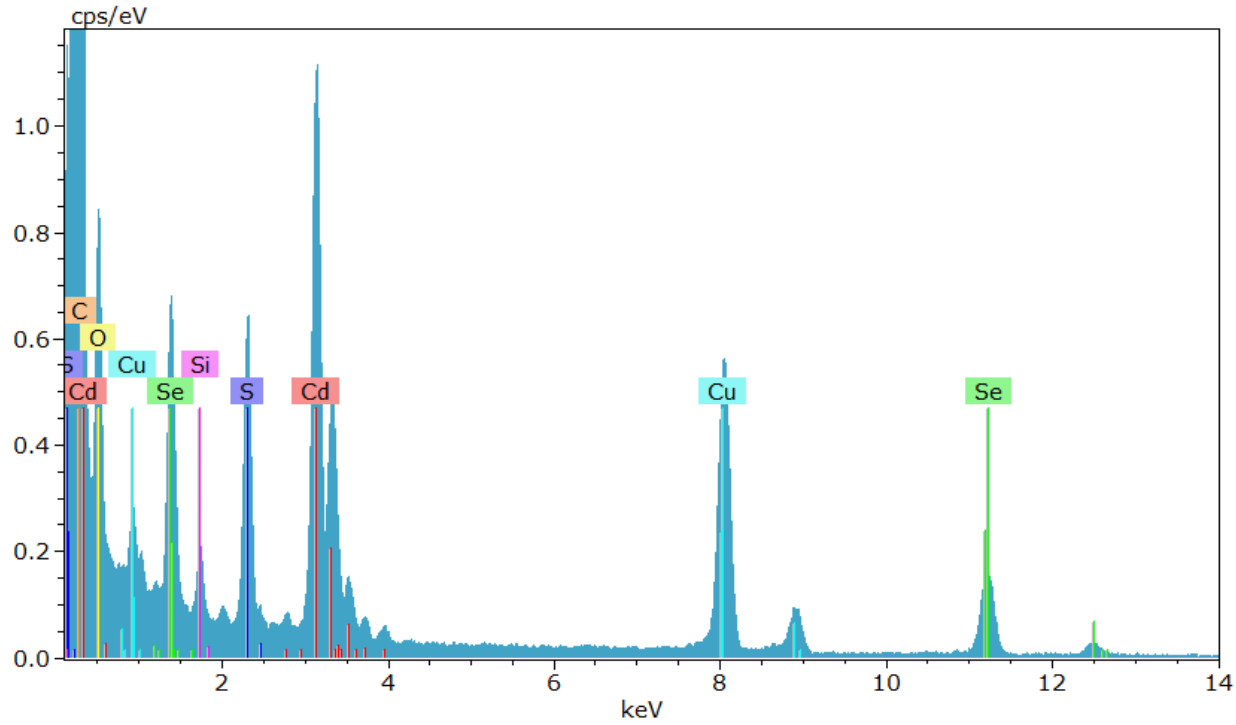

Fig. S7 Wide-window electron-dispersive X-ray spectrum of QD655s.

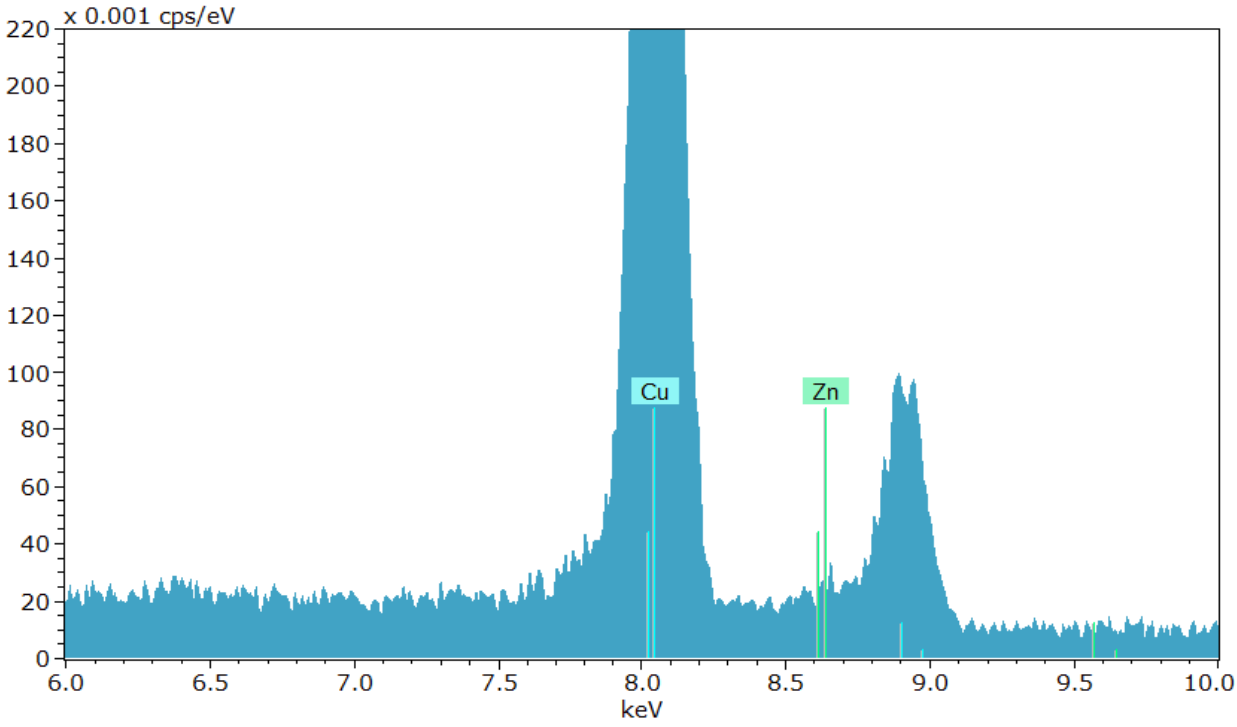

Fig. S8 Narrow-window electron-dispersive X-ray spectrum of QD655s highlighting little to no Zn signal.

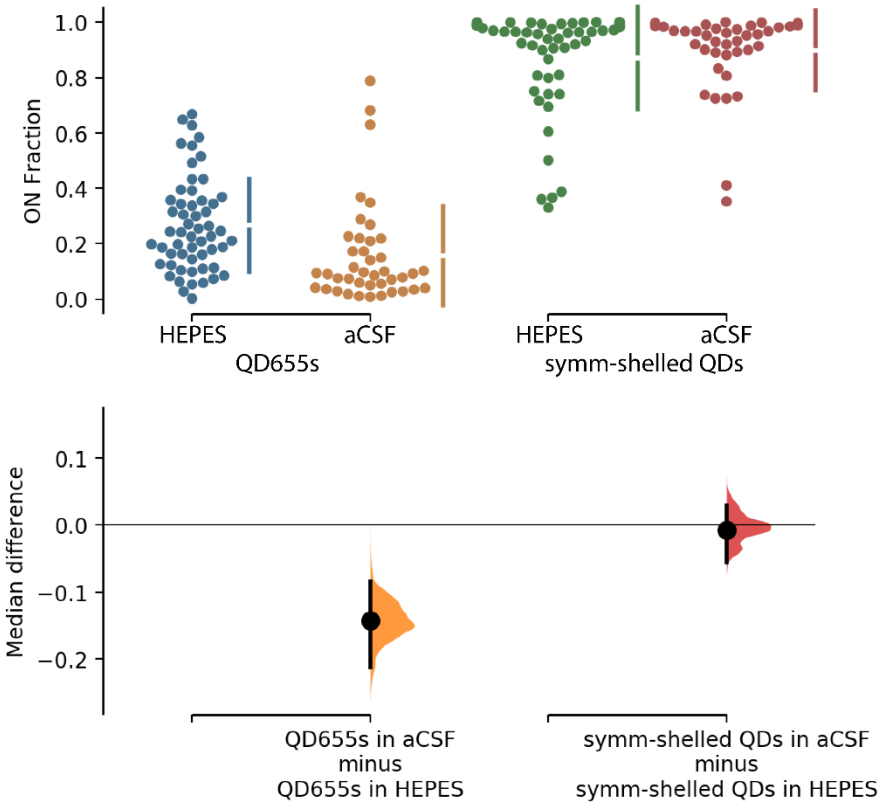

Fig. S9 Data analysis using bootstrap-coupled estimation (DABEST). Distributions of mean differences between QD subgroups displaying estimated effect sizes.

Table S2. Summary of statistics value outputs from data analysis using bootstrap-coupled estimation (DABEST).[8] P-values are outlined in blue.

Control Group	**Test Group**	Median Difference (ON-fraction)	Kruskal-Wallis p-value	Kruskal-Wallis H(2)
QD655s in HEPES	QD655s in aCSF	-0.142	7.32 x 10-5	15.72
symm-shelled QDs in HEPES	symm-shelled QDs in aCSF	-0.00783	0.909	0.013

Supporting Information References

1. O. Chen, J. Zhao, V. P. Chauhan, J. Cui, C. Wong, D. K. Harris, H. Wei, H.-S. Han, D. Fukumura, R. K. Jain and M. G. Bawendi, *Nature Materials*, 2013, **12**, 445.
2. S. M. Wichner, V. R. Mann, A. S. Powers, M. A. Segal, M. Mir, J. N. Bandaria, M. A. DeWitt, X. Darzacq, A. Yildiz and B. E. Cohen, *ACS Nano*, 2017, **11**, 6773-6781.
3. V. R. Mann, A. S. Powers, D. C. Tilley, J. T. Sack and B. E. Cohen, *ACS Nano*, 2018, **12**, 4469-4477.
4. A. D. Dukes, P. C. Samson, J. D. Keene, L. M. Davis, J. P. Wikswo and S. J. Rosenthal, *The Journal of Physical Chemistry A*, 2011, **115**, 4076-4081.
5. N. J. Orfield, J. R. McBride, F. Wang, M. R. Buck, J. D. Keene, K. R. Reid, H. Htoon, J. A. Hollingsworth and S. J. Rosenthal, *ACS Nano*, 2016, **10**, 1960-1968.
6. K. R. Reid, J. R. McBride, N. J. Freymeyer, L. B. Thal and S. J. Rosenthal, *Nano Letters*, 2018, **18**, 709-716.
7. L. B. Thal, D. M. Bailey, O. Kovtun and S. J. Rosenthal, in *Chemical and Synthetic Approaches in Membrane Biology*, ed. A. K. Shukla, Springer New York, New York, NY, 2017, DOI: 10.1007/8623_2016_12, pp. 219-230.
8. J. Ho, T. Tumkaya, S. Aryal, H. Choi and A. Claridge-Chang, *Nature Methods*, 2019, **16**, 565-566.