InAs(ZnCdS) Quantum Dots Optimized for Biological Imaging in the Near-Infrared

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Supporting Information

Materials: Indium acetate (99.99, Alfa Aesar), octadecene (90%, Sigma), myristic acid (99.5%, Sigma), diethyl zinc (Strem), dimethyl cadmium (Strem), bis(trimethylsilyl)sulfide (Fluka), tris(trimethylsilyl)arsine (Nanomeps, France), tri-n-octylphosphine (97%, Strem), oleyl amine (Acros Organics), and all other reagents were obtained from Sigma. All solvents were spectrophotometric grade and purchased from EMD Biosciences. Oleyl amine was degassed under vacuum at 120 ^{o}C for two hours prior to use. Dimethyl cadmium and diethyl zinc were passed through a 20 nm filter prior to use. QD synthesis was performed using standard air free techniques on a nitrogen filled schlenk line. All glove-box manipulations were performed in an mBraun box (<0.1ppm oxygen) or VAC box (<0.5 ppm oxygen).

InAs Core Synthesis: Prepared similarly as previously reported by Xie and Peng.[1] 0.15 mmol of indium acetate, 0.45 mmol of myristic acid, and 3 ml of octadecene were added to a four neck 25 ml round bottom flask. The solution was heated to 100 ^{o}C under vacuum for one hour. The reaction mixture was then placed under nitrogen and heated to 150 ^{o}C. A solution of 0.125 mmol of tris(trimethylsilyl)arsine and 0.75 ml of tri-n-octylphosphine was prepared (under minimal lighting) in a nitrogen filled glovebox. This solution was then swiftly injected into the reaction flask and the temperature was increased to 230 ^{o}C for twenty minutes.

InAs(ZnCdS) Synthesis: After the synthesis of the InAs cores, the reaction flask was cooled to 170 ^{o}C and 3 ml of dry oleyl amine was injected (addition of over-coating precursors began immediately after the injection of amines). For shell growth, solution A was prepared by adding 1 mmol of bis(trimethylsilyl)sulfide to 10 ml of tri-n-octyl phosphine. Solution B was prepared by adding 0.34 mmol of dimethyl cadmium and 0.66 mmol of diethyl zinc to 10 ml of tri-n-octyl phosphine. Solution A and B were added simultaneously via syringe pump to the reaction flask at the rate of 1.5 ml/hour. The reaction was stopped when the peak photoluminescence reached ~800 nm.

CdSe(CdS) Synthesis: CdSe cores were synthesized according to previously reported procedures[2-4] and were overcoated with a CdS shell. For pure CdS shells, we developed a successive ion layer adsorption and reaction (SILAR) procedure that is modified from those reported by Peng et al and Mews et al.[5,6] Briefly, CdSe cores with a first exciton feature at 491 nm were synthesized by heating a mixture of trioctylphosphine (TOP), trioctylphosphine oxide (TOPO), CdO (0.9 mmol), and tetradecylphosphonic acid (TDPA, 2.0 mmol) to 340 °C under nitrogen, removing evolved water *in vacuo* at 160 °C, re-heating to 360 °C under nitrogen, and rapidly introducing trioctylphosphine selenide (TOPSe, 3.4 mmol) in trioctylphosphine (TOP), followed by cooling to room temperature. Cores isolated by repeated precipitations from hexane with acetone were brought to 180 °C in a solvent mixture of oleylamine (3 mL) and octadecene (6 mL). Aliquots of Cd and S precursor solutions were then introduced alternately

starting with the metal (Cd), waiting 15 min between the start of each addition. The Cd precursor consisted of 0.6 mmol Cd-oleate and 1.2 mmol decylamine in a solvent mixture of octadecene (3 mL) and TOP (3 mL). The S precursor consisted of 0.6 mmol hexamethyldisilathiane [$(TMS)_2S$] in 6 mL TOP. The dose of each over-coating precursor aliquot was calculated to provide a single monolayer of ions to the QD surface. Addition of a total of 4 aliquots each of Cd and S yielded QDs with emission at 562 nm and a QY close to unity when diluted in hexane.[7]

Synthesis of polymer ligands: The synthesis of the poly(PEG_{12})-PIL and poly($aminoPEG_{11})_{25\%}$-PIL ligands are reported in detail elsewhere.[7]

Ligand exchange with poly(PEG_{12})-PIL: QDs (2 nmol) were precipitated 1x using a mixture of acetone and butanol and brought into 50uL of $CHCl_3$. The QD stock solution was added to a solution of poly(PEG)-PIL (5 mg) in $CHCl_3$ (30uL), and stirred for 10 min at RT, after which 30 uL of MeOH was added followed by stirring for an additional 40 min. QD samples were precipitated by the addition of EtOH (30 µL), $CHCl_3$ (30 µL), and excess hexanes. The sample was centrifuged at 4000 g for 2 min, the supernatant discarded, and the pellet precipitated once more by the addition of EtOH, $CHCl_3$, and excess hexanes. After centrifugation and removal of the supernantant, the pellet was dried *in vacuo*, and PBS (500 µL, pH 7.4) was added, followed by filtration through a 0.2 µm filter.[7]

Covalent conjugation of streptavidin to poly($aminoPEG_{11})_{25\%}$. Commercial SA (50 uL, 10 mg/mL) was activated in MES buffer (pH 6.5) using Sulfo-NHS and EDC (20 eq.) for 20 min at RT. The activated SA was mixed with poly($aminoPEG_{11})_{25\%}$ QDs in sodium bicarbonate buffer at pH 8.4 at a SA:QD ratio of 5:1 and allowed to react for 1 hr. The samples were dialyzed 2x though a 50kDa MW cut-off spin concentrator and then used for labeling experiments.[7]

Quantum Yields: The QY of 800 nm emitting InAs(ZnCdS) QDs was measured relative to 1,1′,3,3,3′,3′-hexamethylindotrycarbocyanine iodide (HITC, QY = 26%) (λ_{ex} = 680 nm). Solutions of QDs in PBS and dye in methanol were optically matched at the excitation wavelength. QYs were calculated from the following expression: $QY_{QD} = QY_{Dye}$ x ($Absorbance_{dye}$ / $Absorbance_{QD}$) x ($Peak\ Area_{QD}$ / $Peak\ Area_{Dye}$) x $(n_{QD\ solvent})^2/(n_{Dye\ solvent})^2$.

Transmission Electron Microscopy (TEM): TEM measurements were performed on a JEOL200CX microscope. The InAs(ZnCdS) QDs in water were dropped onto a Ted Pella ultra-thin carbon type A grid.

Wavelength Dispersive Spectroscopy (WDS): Samples were prepared by precipitating the QDs from solution three times with a mixture of hexanes, butanol, and acetone. The QDs were then drop-cast onto doped silicon substrates and analyzed on a JEOL 8200 scanning electron microscope.

Gel Filtration Chromatography (GFC): GFC was performed using an ÄKTAprime Plus chromatography system from Amersham Biosciences equipped with a self-packed Superdex 200 10/100 column. PBS (pH 7.4) was used as the mobile phase with a flow rate of 1.0 mL/min. Detection was achieved by measuring the absorption at 280 nm.

Cell Labeling: HeLa cells were grown in DMEM (Mediatech) with 10% Fetal Bovine Serum (Invitrogen), 50 U/mL penicillin and 50 µg /mL streptomycin (Invitrogen). Transfection plasmids were a

kind gift from A.Ting (MIT, US). The cells were transfected using 1 µl Lipofectamine 2000 (Invitrogen), 0.2 µg of BirA-ER and 0.2 µg of AP-YFP-TM per well of an 8-well chamber slide (LabTek). 1 mM biotin was added to the media during plasmid expression. Cells were imaged under 4°C PBS the day after transfection. 1% Bovine Serum Albumin (Sigma) was added to block non-specific binding during specific binding studies of ligand-coated quantum dots. Commercial BSA is known to contain biotin, and the stock BSA solution was dialyzed with a 3 kDa cutoff dialysis tube three times for 8 h in PBS pH 7.4, in 4°C. [7-10]

Intravital Microscopy: *In vivo* imaging was carried out using a custom-built multiphoton microscope (Olympus) with a Ti:Sapphire excitation laser at 850 nm (Mai-Tai HP, Spectra-Physics) and a 20x 0.95 NA water-immersion lens (Olympus). Mammary fat pad window chambers were implanted in female SCID mice as described previously E0771 mammary tumors were implanted in the mammary fat pad in these chambers, and were allowed to grow for two weeks until the tumors were roughly 3 mm in diameter and well-vascularized. [11] To determine appropriate concentrations for equal photoluminescence intensity for green visible CdSe(CdS) and NIR InAs(ZnCdS) QDs in solution, multiphoton imaging of mixed solutions of these QDs in glass microslides (VitroCom) was conducted. A mixture at these concentrations was then prepared, and 200uL of this solution was injected intravenously into the mouse via a bolus retro-orbital injection. An image mosaic was then collected for the entire tumor, at depths from 0-200 um into the tissue.

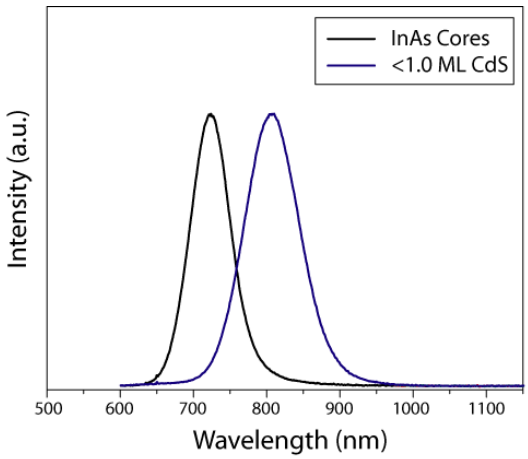

Figure S1. Photoluminescence of InAs(CdS) QDs where the QDs peak photoluminescence has already shifted beyond the targeted 800 nm window, when only a sub-monolayer (<1.0 ML) of CdS has been grown.

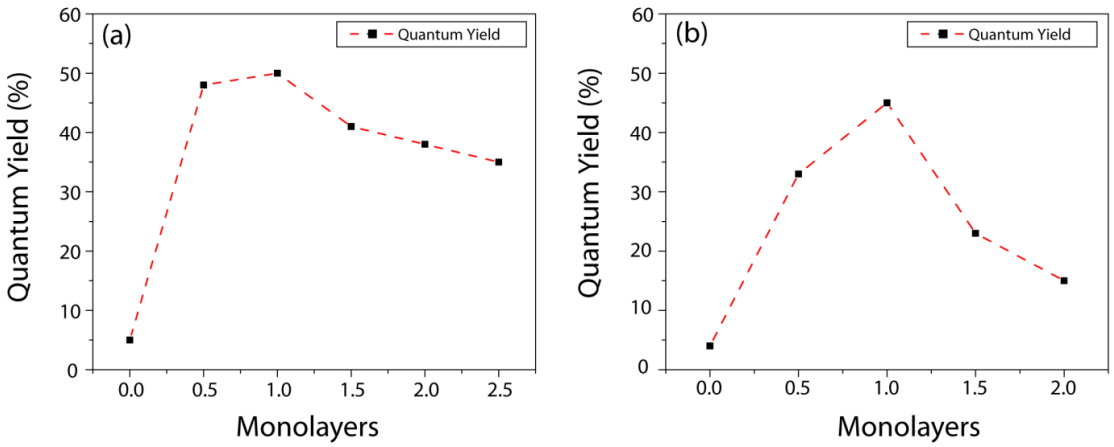

Figure S2. (a) Quantum yields as a function of ZnCdS shell growth on InAs cores with the addition of oleyl amine and (b) the growth of a ZnCdS shell without the addition of oleyl amine. The decrease in QY in (b) is likely the result of decreased surface passivation as residual $In(Myr)_3$ is consumed by the highly reactive over-coating precursors.

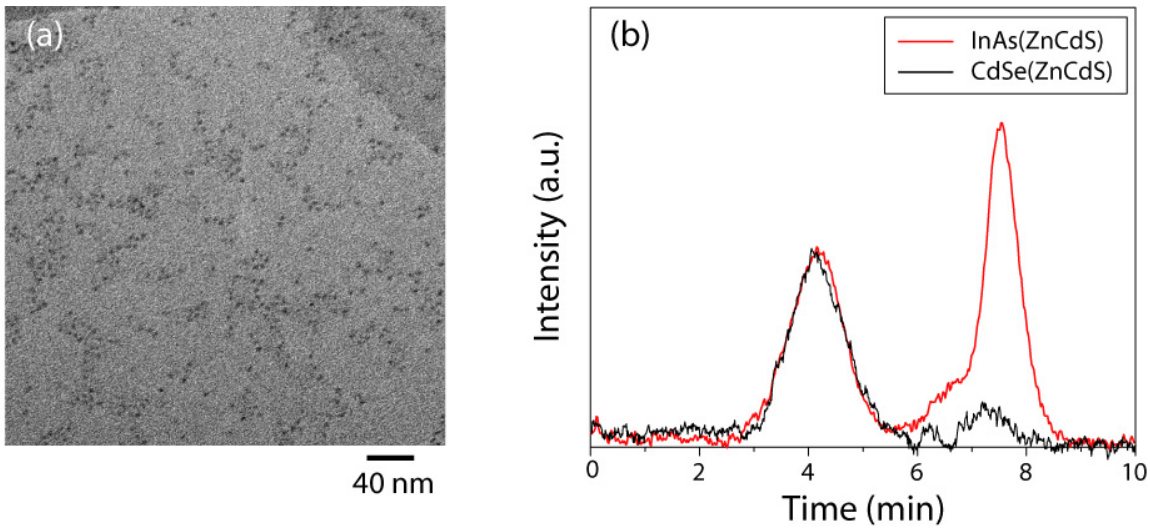

Figure S3. (a) TEM of InAs(ZnCdS) QDs with inorganic size ~2.9 nm drop-cast from water. (b) GFC of InAs(ZnCdS) poly(PEG_{12})-PIL (red) QDs with a retention time of 4.17 minutes corresponding to a HD of <10 nm (the peak at 7.5 minutes corresponds to free poly(PEG)-PIL ligand) and CdSe(CdS) poly(PEG_{12})-PIL (black) QDs with a retention time of 4.10 minutes corresponding to a HD of <10 nm.

(a) InAs(ZnCdS) Calculated Shell Growth

QD	Size (Diameter)	S/As Ratio
Cores	1.40 nm[1]	--
0.5 ML	1.68 nm	0.7
1.0 ML	1.96 nm	1.8
1.5 ML	2.24 nm	3.1
2.0 ML	2.52 nm	4.9
2.5 ML	2.80 nm	7.0
3.0 ML	3.08 nm	9.6

(b) InAs(ZnCdS) Experimental Shell Growth

QD	Size (Diameter)	S/As Ratio
InAs(ZnCdS)	2.9 +/- 0.3 nm (TEM)	7.1 +/- 0.1 (WDS)

(c) InAs(ZnCdS) WDS Atomic %

In	As	Cd	Zn	S
6.4 +/- 0.2 %	5.6 +/- 0.2 %	15.0 +/- 0.3 %	32.8 +/- 0.7 %	39.8 +/- 0.8 %

Table S1. (a) Calculated values of the sulfur to arsenic (S/As) ratio from 0.0-3.0 monolayers. The ZnCdS lattice parameter was estimated assuming a linear dependence of the lattice parameters with alloy composition, for $Zn_{0.7}Cd_{0.3}S$ a lattice parameter of a = 5.6 Å was estimated. We have defined '1 monolayer' as the addition of 5.6 Å to the inorganic diameter of the QD. (b) The experimental values (with rms standard deviations) for the inorganic diameter of InAs(ZnCdS) by TEM and the S/As ratio as determined by wavelength dispersive spectroscopy (WDS). (c) Average of five WDS measurements (with rms standard deviations) for the elemental composition of the as synthesized InAs(ZnCdS) QDs.

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