

Electronic Supplementary Information For:

Gel permeation chromatography as a multifunctional processor for nanocrystal purification and on-column ligand exchange chemistry

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Experimental Techniques

Materials and methods. The following chemicals were used as received unless otherwise specified. Cadmium oxide (CdO; 99.999%), Zinc oxide (ZnO; 99.999%), Trioctylphosphine (TOP; 97%), Trioctylphosphine oxide (TOPO; 99%) and 4-Cyano-4- [(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid were purchased from STREM Chemicals. Oleic Acid (99%), 1- Octadecene (ODE; 90% technical grade), 1-Tetradecylphosphonic Acid (TDPA; 98%), Selenium (Se; 99.999%), octanethiol (98%) and tri-n-hexylamine were purchased from Alfa Aesar. Bio-Beads S-X1 GPC medium was obtained from Bio-Rad Laboratories, Inc. Toluene-d₈ (D, 99.5%), pyridine-d₅ (D, 99.5%), chloroform-d (D, 99.5%) and Tetrahydrofuran-d₈ (THF; D, 99.5%) were obtained from Cambridge Isotope Laboratories, Inc. Decylamine (95%) was purchased from Sigma Aldrich. Oleylamine (80-90%) and Bis(trimethylsilyl) sulfide ((TMS)₂S; 95%) were purchased from Acros Organics. Rhodamine Chloride 590 (R590, MW 464.98) was obtained from Exciton. Toluene (99.5% ACS analysis grade) was purchased from Mallinckrodt Chemicals. 200 Proof Ethyl Alcohol (Ethanol) was obtained from Decon Laboratories, Inc. Acetone (99.9%) was purchased from VWR. AIBN was purchased from Sigma Aldrich and recrystallized thrice from methanol. Poly(ethyleneglycol) methacrylate (475 g mol⁻¹) was obtained from Sigma Aldrich and passed through a neutral alumina column to remove inhibitors before use. P(SiMe₃)₃ was prepared following literature procedures.¹ Indium acetate (99.99%) and myristic acid (≥99%) were purchased from Sigma-Aldrich and used as received. All the other chemicals were purchased from Fisher and used as received. Synthetic or analytical procedures performed under nitrogen (N₂) or vacuum environment were carried out using either Schlenk line techniques or a glovebox.

Optical spectroscopy. The optical absorption spectrum was recorded using a Thermo Scientific Evolution Array UV-Visible Spectrophotometer. Routine emission spectra were recorded by an Ocean Optics USB 4000 spectrometer under ~365 nm excitation.

NMR analysis of QDs. The spectra were recorded on Bruker Avance III 400 or Avance III 300 HD. The quantitative ¹H NMR spectra were measured with ferrocene as the internal standard and 30 s relaxation delay, allowing the system to reach a reliable equilibrium². The ³¹P NMR spectra of QD samples were measured with 512 scans to increase the signal-to-noise ratio.

Transmission electron microscopy imaging of the NCs. All images were recorded with a Hitachi H-8000 microscope except the high-resolution images of InP NCs, which were recorded by JEOL 2100F 200 kV aberration-corrected FEG-STEM/TEM. The samples were made by drop casting from the dilute solution (if the solvent is water, the excess solution was wicked away with a tissue after 30 min deposition). Before imaging, the grids were pumped dry under vacuum for 2 hours.

Thermogravimetric Analysis (TGA). Samples were prepared by concentrating QD solutions under vacuum and then transferring them to the platinum pan in liquid form. TGA was conducted on a TA Instruments Q5000, with a heating rate of 10 °C/min, from room temperature to 650~750 °C under constant nitrogen flow.

Energy-dispersive X-ray spectroscopy (EDX) analysis. The EDX spectra were measured by Tescan Vega3 SBU SEM coupled with a Thermo EDX detector. The sample chips were made by drop casting the solution onto a 0.7×0.7 cm² Si chip at 60°C. The accelerating voltage was 10 kV.

Synthesis of CdSe QDs. TDPA-capped CdSe QDs were synthesized by the well-known hot injection method. In a representative synthesis, CdO (90 mg) was heated with TDPA (430 mg) at 330°C in a solvent consisting of TOP (4.5 mL) and TOPO (4.5 g) under nitrogen flow until the solution became colorless. Following removal of evolved H₂O under vacuum at 130°C, the solution was heated again to 360°C under

nitrogen. As-prepared TOPSe (0.96 mL, 2.2 M) was injected rapidly into the reaction pot, which was immediately cooled down to room temperature by a blower. The Cd:TDPA:Se molar ratio is 1:2.2:3. The sample was precipitated by acetone/methanol and redispersed in hexane. The solution was then transferred to freezer for 2 hours and centrifuged to remove the excess TOPO. Hexane was then removed by vacuum and the QDs were redissolved in toluene (THF or pyridine).

OA-capped CdSe QDs were synthesized by a similar method. CdO was mixed with oleic acid in 1-octadecene to generate cadmium oleate. TOPSe was injected into the cadmium oleate solution at 280°C and the reaction was quenched immediately by cooling down the temperature with a blower. The Cd:OA:Se molar ratio is 1:2.2:2. The sample was precipitated by acetone/methanol and redispersed in toluene.

Synthesis of thiol-capped Au NCs. The thiolate-capped Au nanoparticles (NPs) were prepared by a two phase liquid-liquid synthesis method designed by Mathias Brust and co-workers³, but with tetrabutylammonium bromide as the phase transfer agent. Briefly, tetrachloroaurate (AuCl_4^- , 25 μmol) was transferred from aqueous solution to toluene by tetrabutylammonium bromide and then the AuCl_4^- was reduced in the presence of dodecanethiol (1.5 mmol) by stirring the toluene solution with aqueous sodium borohydride (~10 mg); the dodecanethiol functions as the ligand for the resulting reddish or black solution of Au nanoparticles.

Synthesis of carboxylate-capped InP QDs. The InP QDs were prepared following a published result.¹ In a typical synthesis, indium acetate and myristic acid were mixed in 1-octadecene at 100°C for approximately 12 h under vacuum to generate the indium myristate solution. The apparatus was then placed under nitrogen and raised to 316°C. $\text{P}(\text{SiMe}_3)_3$ was dissolved in 1-octadecene and injected into the indium myristate solution at 315°C as the temperature controller was set to 285°C. The temperature was maintained at 285°C for the quantum dot growth. After the synthesis, the solvent 1-octadecene was removed under reduced pressure and the samples were redispersed in toluene.

Synthesis of CdSe/CdS, CdSe/CdZnS NCs. The spherical QDs were made by previously described selective ionic adhesion and reaction (SILAR) method.⁴ In short, the purified TDPA-capped CdSe QDs were injected into a mixture of amine (oleylamine for CdS shell growth and trihexylamine for CdZnS shell growth) and 1-octadecene. The metal precursor is either $\text{Cd}(\text{Oleate})_2$ or a mixture of $\text{Cd}(\text{Oleate})_2$ and $\text{Zn}(\text{Oleate})_2$ (the ratio of Cd:Zn is 3:7), and the sulfur precursor is $(\text{TMS})_2\text{S}$. The growth temperature is 200°C. The volume of each injection is calculated to apply 0.8 monolayers each cycle with metal dosing first. A total of 4 monolayers were coated onto the CdSe cores. After the coating process, the particles were precipitated by acetone and redispersed in toluene.

Synthesis of CdSe/CdS Nanorods. The CdSe/CdS nanorods were synthesized following the procedure described by Carbone *et al.*⁵ The Cd precursor for CdSe/CdS_NC_2 (**Figure 2B**) was a mixture of Cd phosphonate in TOPO (92 mg CdO, 291 mg octadecylphosphonic acid and 80 mg hexylphosphonic acid dissolved in 3 g TOPO). After heating the Cd precursor solution to 370°C, a mixture of 100 nmol phosphonate-capped CdSe QDs in TOP and S precursor (60 mg S dissolved in 1.5 g TOP) was injected into the solution. The temperature was kept at above 350°C for 10 minutes and then cooled down to the room temperature. CdSe/CdS_NC_3 (**Figure 2C**) was prepared in the same method with higher amount of HPA (101 mg). CdSe/CdS_NC_4 (**Figure 2D**) was prepared with lower dose of CdSe seeds (60 nmols). The absorption spectra, TEM images of the representative nanorod samples (CdSe/CdS_NC_4) are shown in **Figure S6**. The nanorods were precipitated with methanol and redispersed in toluene.

GPC column packing. To pack the preparative column, 4 g of Bio-Beads were first swollen in toluene (THF, pyridine) overnight (for the thiol *in situ* GPC ligand exchange study, 6 g of Bio-Beads were used). Clean solvent (5 mL) was placed in the glass column (inner diameter ~1 cm) with a filter (0.2 μm pore size

filter and glass wool) and a Teflon valve. All of the swollen beads were transferred to the column. After the gel settled down and formed the column with a height of approximately ~30 cm, a small layer of sand was carefully placed at the top of the column and the pure solvent was used to rinse the column until no free polystyrene was present in the eluent (tested by UV-Vis absorption). The chloroform column was prepared by rinsing chloroform through the packed toluene column until the solvent being completely switched to chloroform.

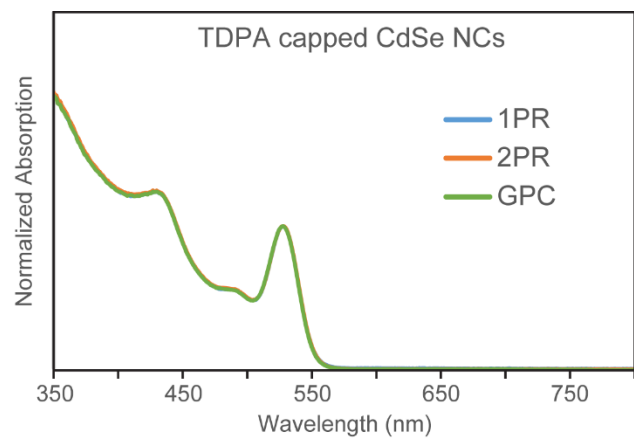
Synthesis of polymeric imidazole ligands (PILs). The PILs were prepared as described previously.⁶ Poly(PEGMA-co-NMS) was made by mixing poly(ethylene glycol) methacrylate (PEGMA, 1.5 mmol), N-methacryloxysuccinimide (NMS, 1.3 mmol), 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA, 0.025 mmol), and 50 mg trioxane (as NMR standard) in 1 mL DMF. The reaction was activated by 25 μ L 0.1 M AIBN and ran at 70°C for 7 hours under N₂. After the reaction, the polymer was purified to remove the RAFT reagent with AIBN, and then mixed with excess amount of histamine dihydrochloride in triethylamine / anhydrous DMSO solution for a post-modification at 70°C for 24 hours. The as-synthesized PILs were dialyzed and freeze-dried. The number averaged molecular weight of the PILs is 27 kD with a polydispersity index (PDI) of 1.39. The ratio between PEG to the imidazole group is close to 1:1.

***In situ* ligand exchange reactions.** For the reaction between oleate-capped CdSe QDs and octanethiol, 30 nmols of dots were used to react with 0.1 g thiol ligands. The total volume of the toluene GPC column is close to 40mL. First the thiol ligands were dissolved in 10 mL toluene and then loaded onto the column. After that, 6mL pure toluene was introduced and then 30 nmol unpurified QDs in 0.7 mL toluene was injected. The sample was rinsed out by toluene. The flow rate for this reaction is 0.4 mL/min. A similar experiment (30nmol QDs mixed with 0.1 g octanethiol in 0.7 mL toluene) was performed on the bench, and the reaction was quenched (reaction time is close to 15 min) by precipitation with acetone and methanol.

For the reaction between TDPA-capped CdSe QDs and pyridine, 50 nmols of dots in 0.5 mL pyridine was injected into a pyridine column. The flow rate of the column is around 0.3 mL/min. Another 50 nmols of dots were stirred in 1 mL pyridine vigorously for 45 min as a comparison. The exchanged phosphonate ligands were removed by precipitation with hexane and the sample was redispersed in toluene.

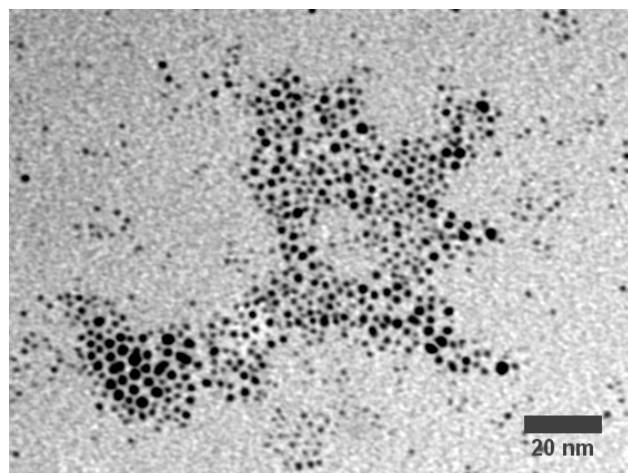
For the reaction between oleate-capped CdSe/CdZnS QDs and PILs, 10 nmols of unpurified dots were used to react with 500 nmols of 27 kD MA-PILs described previously. The mixture of the polymer and QDs were injected into a chloroform GPC column (flow rate is 1 mL/min and total volume is 28 mL) and eluted out around 10 min.

Figure S1



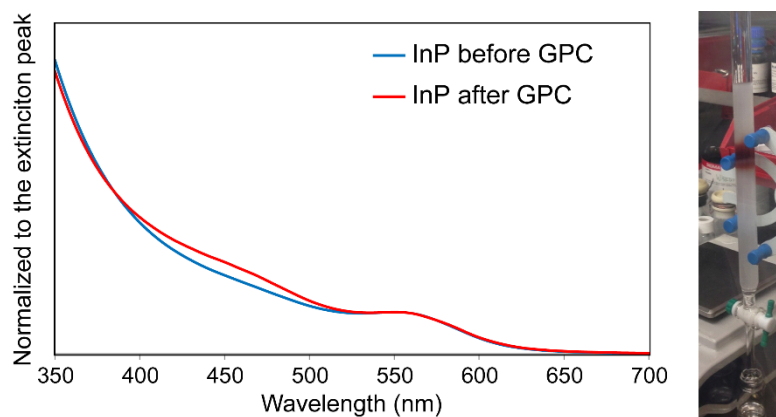
Absorption spectra of TDPA-capped CdSe NCs purified by different methods.

Figure S2



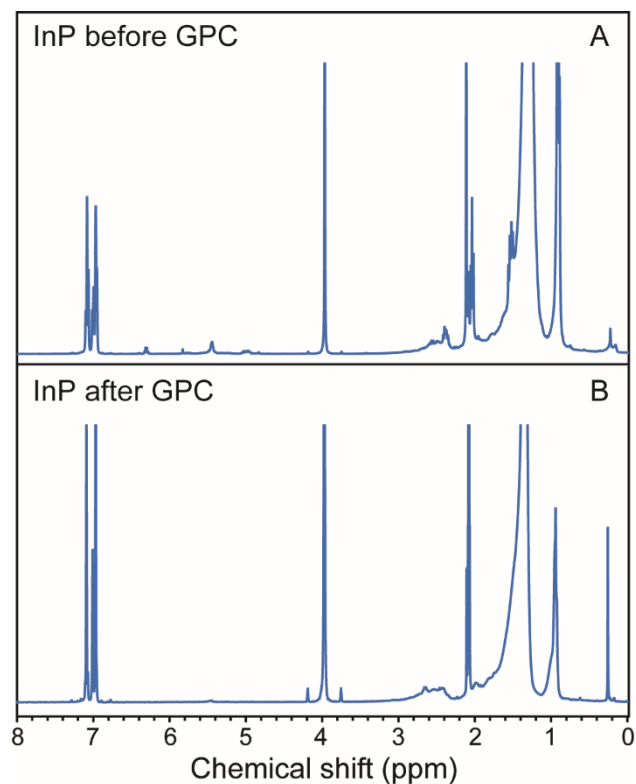
TEM image of the thiol-capped Au NCs.

Figure S3



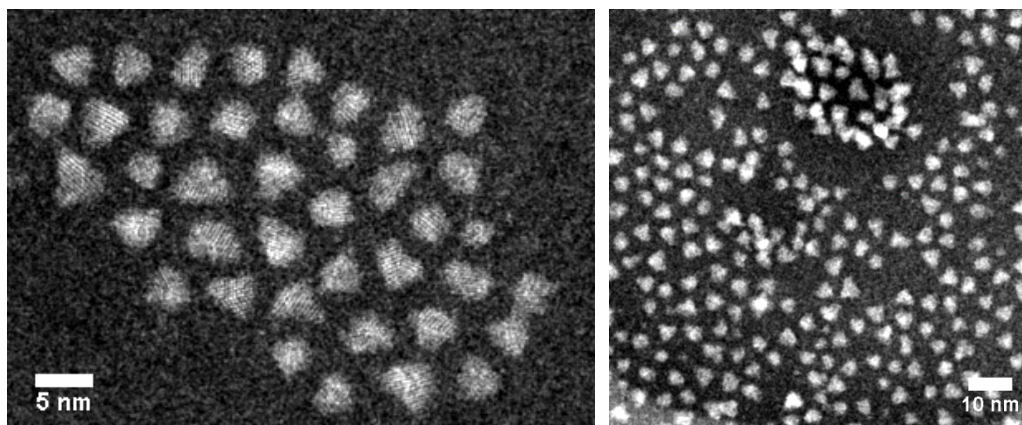
Left: The absorption spectra of the carboxylate-capped InP NCs before and after GPC purification. Right: Photograph of InP NCs transiting a GPC column set up inside a nitrogen-filled glovebox.

Figure S4



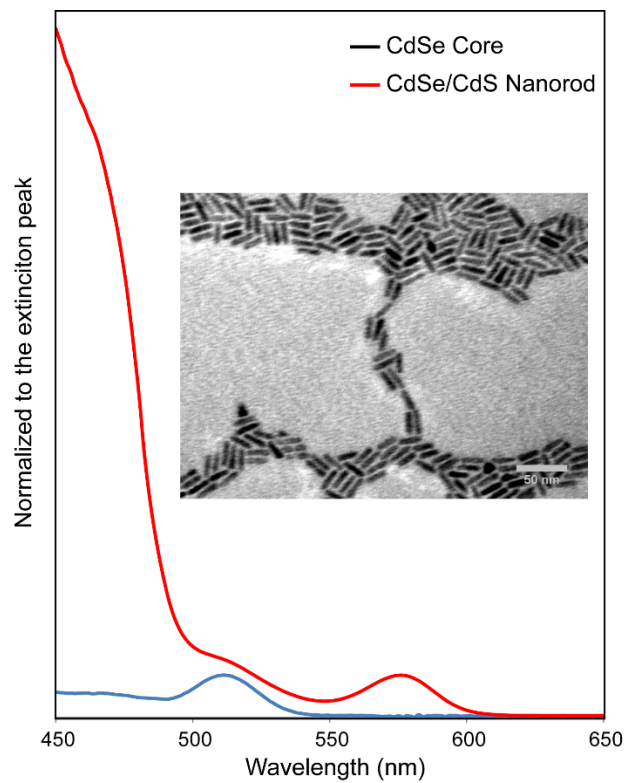
Quantitative ¹H NMR spectra of the carboxylate-capped InP NCs before (A) and after (B) GPC purification with ferrocene as the internal standard.

Figure S5



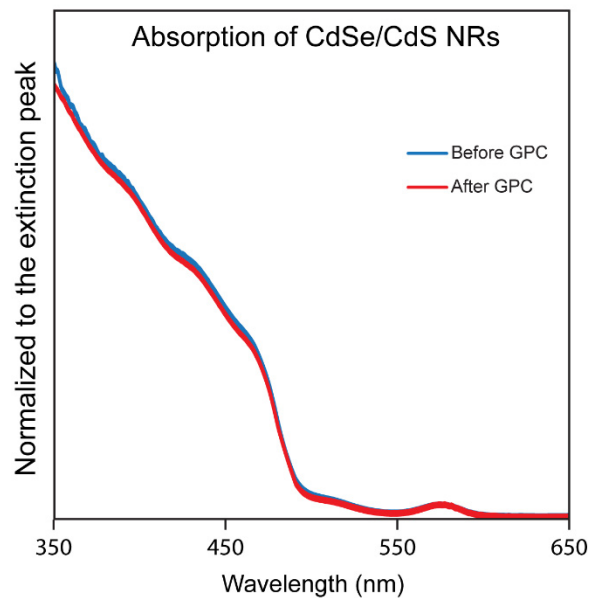
High resolution Z-contrast STEM images of the carboxylate-capped InP NCs after GPC purification. The removal of the excess hydrocarbon ligands on the surface improves the overall quality of the images and does not cause aggregation.

Figure S6



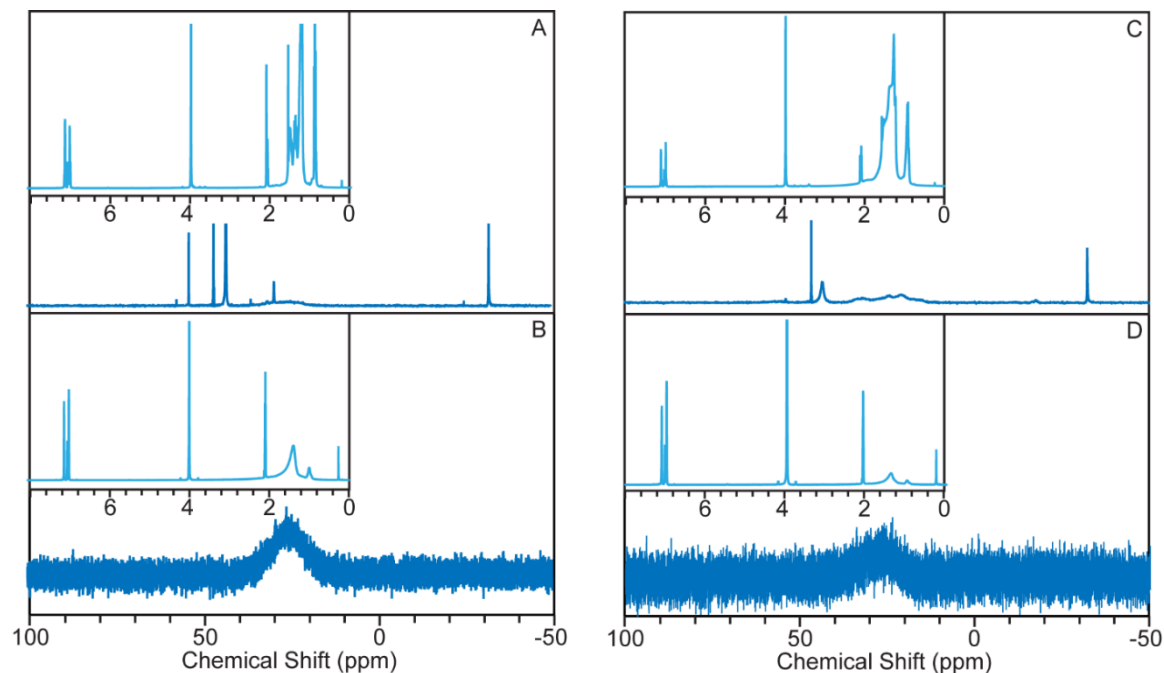
The absorption spectra showing the seeded growth of the nanorods with the TEM image of the nanorods as the inset.

Figure S7



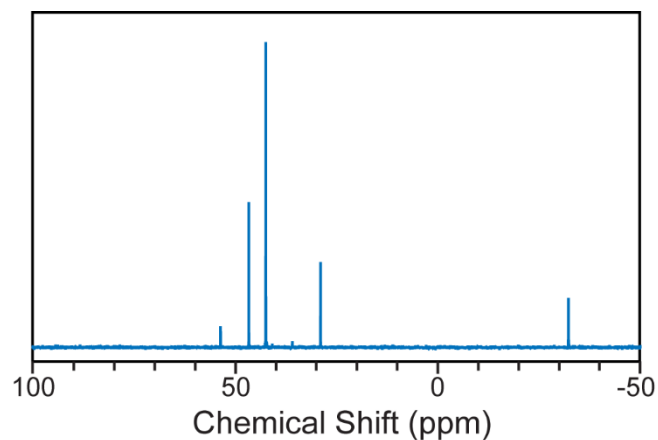
Absorption spectra of the CdSe/CdS nanorods before and after the GPC purification.

Figure S8



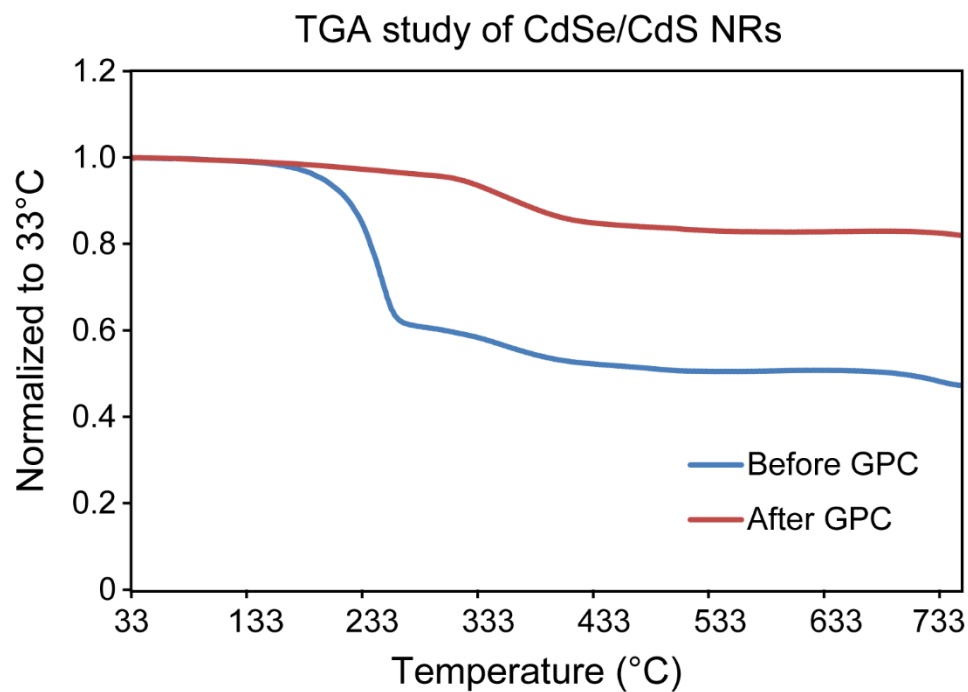
Purification of CdSe/CdS nanorods. ^{31}P NMR of the CdSe/CdS_NC_3 (A,B) and CdSe/CdS_NC_4 (C,D) before (A,C) and after (B,D) the GPC purification with ^1H NMR spectra shown as the insets.

Figure S9



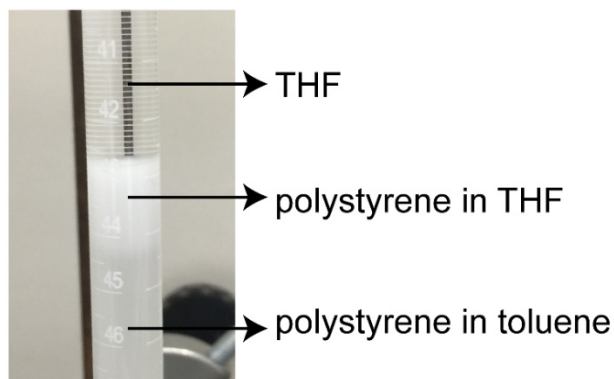
A ^{31}P NMR spectrum of a sample taken at an elution volume close to the total volume of the column reveals impurities that were separated during CdSe/CdS nanorod GPC purification.

Figure S10



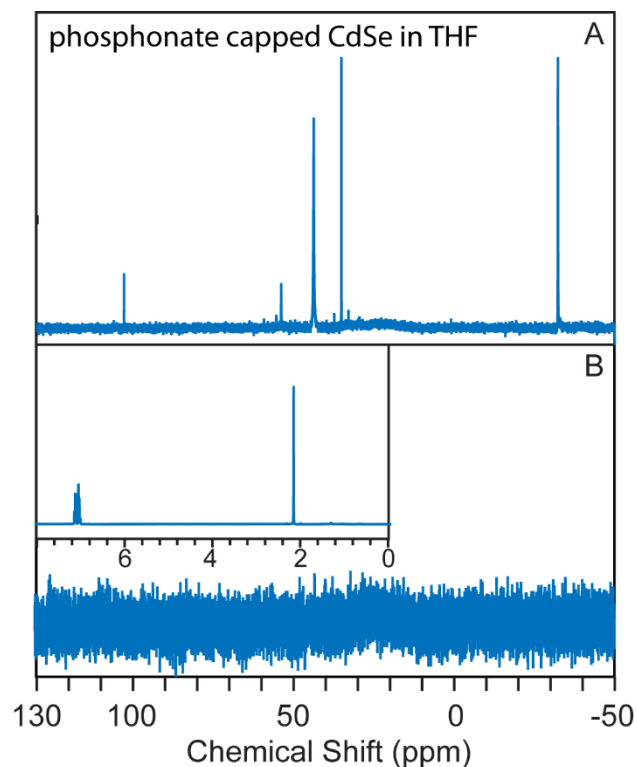
TGA curves of the CdSe/CdS_NC_2 nanorods before and after the GPC purification.

Figure S11



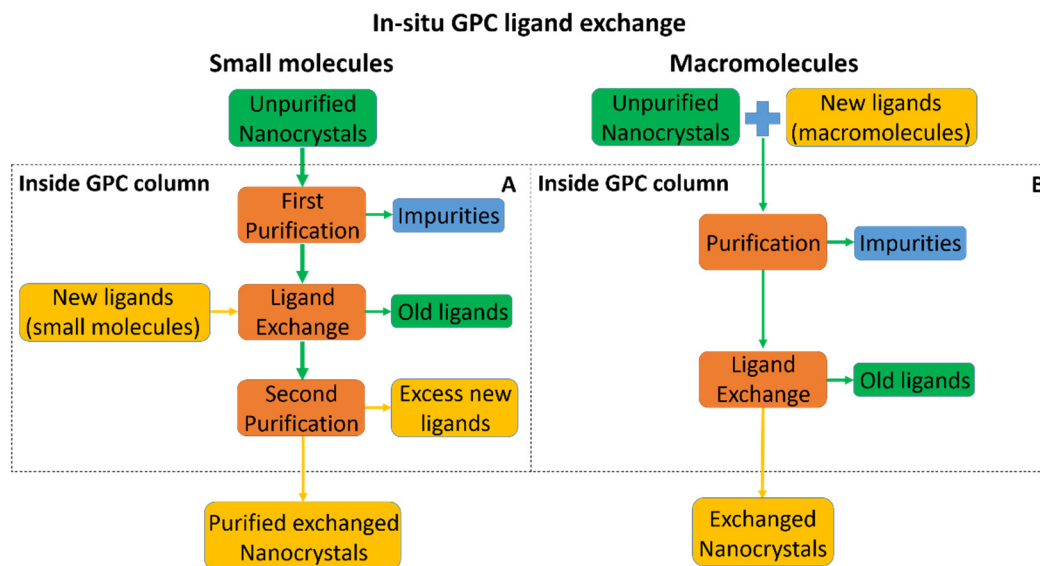
Photograph illustrating the flow of THF inside the toluene column. The dividing line can be easily observed due to the contrast between the polystyrene beads in toluene and THF.

Figure S12



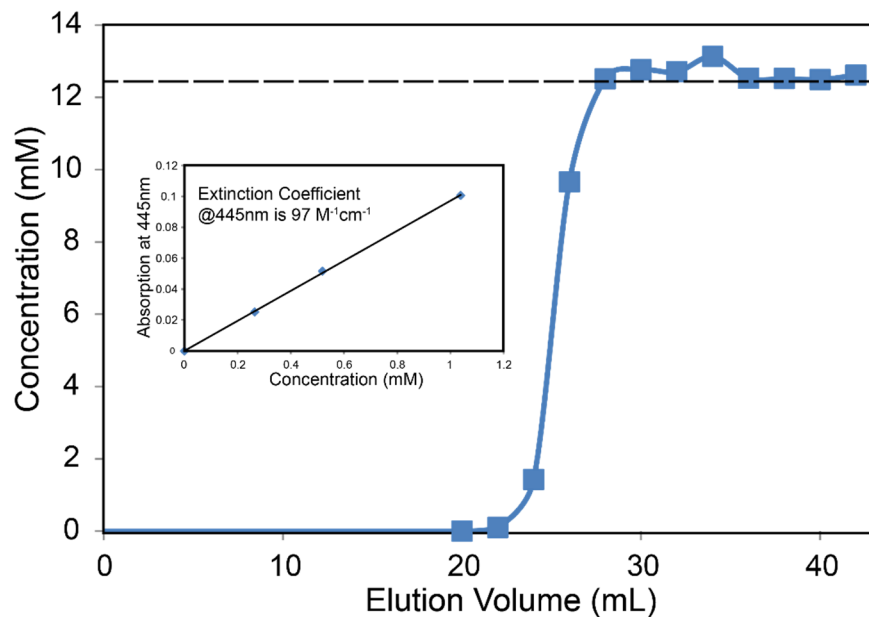
GPC *in situ* solvent change study with TDPA-capped CdSe NCs. (A) ^{31}P NMR of the as-synthesized TDPA-capped CdSe NCs in THF; (B) ^{31}P NMR of the sample after traveling through the toluene column revealing the purification of the NCs; The inset shows the ^1H NMR of the solvent eluted out with the purified NCs, which confirms that the solvent has been changed from THF to toluene.

Figure S13



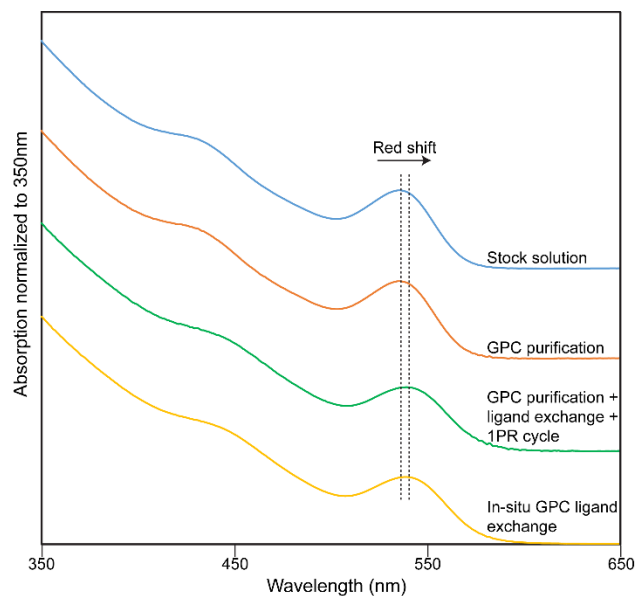
Flow chart of GPC *in situ* ligand exchange illustrating the sequential steps shown in Scheme 1.

Figure S14



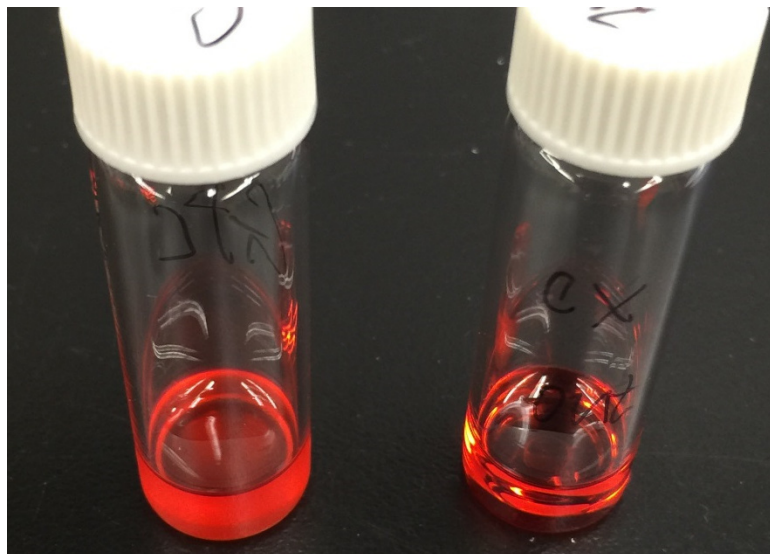
Flow behavior study of the ferrocene solution inside the GPC column. The dashed line indicates the initial concentration of the ferrocene solution. The total volume of the column is around 28 mL. These results confirmed that the elution rate of the small molecules is similar to the flow rate of the column.

Figure S15



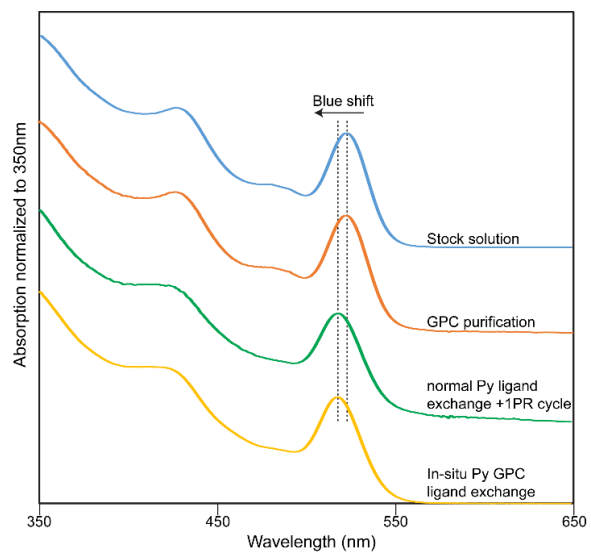
In situ GPC ligand exchange study of oleate-capped QDs with 1-octanethiol. The red shift of the first extinction peak in the exchanged QD samples is an indication of the successful functionalization of the NCs with the thiol ligands.

Figure S16



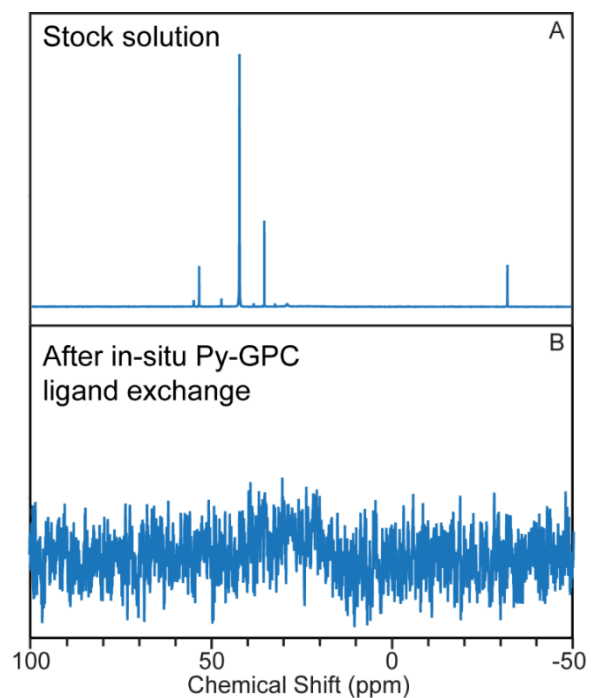
Photographs of octanethiol-exchanged samples prepared by GPC *in situ* ligand exchange method (left) and normal benchtop exchange method (right) after 12 h storage at 4°C (in the dark, ambient air); the precipitation shown in the left sample demonstrates that the *in situ* GPC ligand exchange process is more efficient in removing the excess new ligands.

Figure S17



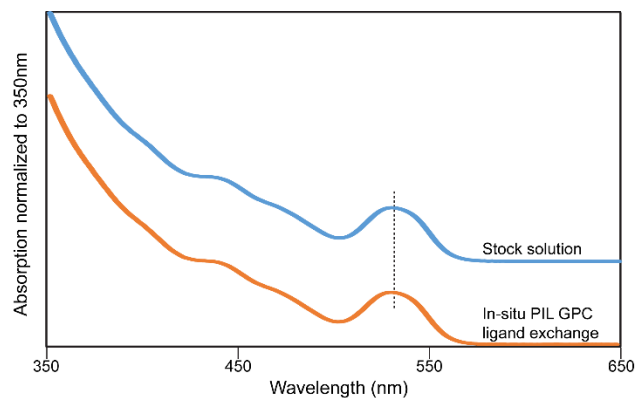
In situ GPC ligand exchange study of TDPA-capped CdSe QDs with pyridine. The blue shift of the first extinction peak is an indication of the successful functionalization of the NCs with pyridine (Py) ligands.

Figure S18



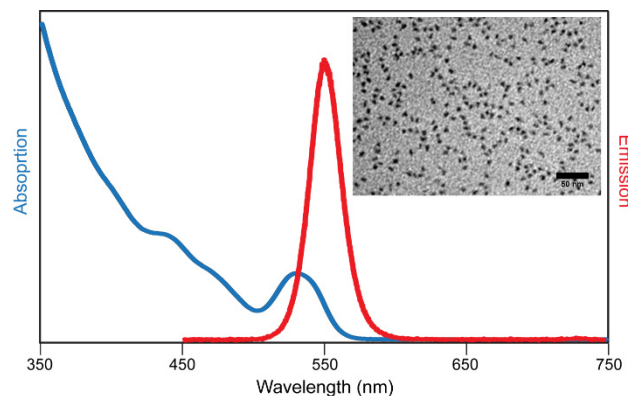
NMR spectra of the TDPA-capped CdSe NCs before (A) and after (B) the *in situ* pyridine (Py) GPC ligand exchange reaction.

Figure S19



Absorption spectra of (initially) oleate-capped CdSe/CdZnS NCs before and after the *in situ* GPC PIL ligand exchange reaction.

Figure S20



Absorption and emission spectra of aqueous PIL-capped CdSe/CdZnS NCs prepared by *in situ* GPC ligand exchange. The inset shows a TEM image of QDs of deposited from this solution, which confirms there is no aggregation during the exchange reaction.

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