ChemComm

FEATURE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2017, **53**, 827

Purification technologies for colloidal nanocrystals

Yi Shen,†‡[a] Megan Y. Gee‡[a] and A. B. Greytak*[ab]

Almost all applications of colloidal nanocrystals require some type of purification or surface modification process following nanocrystal growth. Nanocrystal purification – the separation of nanocrystals from undesired solution components – can perturb the surface chemistry and thereby the physical properties of colloidal nanocrystals due to changes in solvent, solute concentrations, and exposure of the nanocrystal surface to oxidation or hydrolysis. For example, nanocrystal quantum dots frequently exhibit decreased photoluminescence brightness after precipitation from the growth solvent and subsequent redissolution. Consequently, purification is an integral part of the synthetic chemistry of colloidal nanocrystals, and the effect of purification methods must be considered in order to accurately compare and predict the behavior of otherwise similar nanocrystal samples. In this Feature Article we examine established and emerging approaches to the purification of colloidal nanoparticles from a nanocrystal surface chemistry viewpoint. Purification is generally achieved by exploiting differences in properties between the impurities and the nanoparticles. Three distinct properties are typically manipulated: polarity (relative solubility), electrophoretic mobility, and size. We discuss precipitation, extraction, electrophoretic methods, and size-based methods including ultracentrifugation, ultrafiltration, diafiltration, and size-exclusion chromatography. The susceptibility of quantum dots to changes in surface chemistry, with changes in photoluminescence decay associated with surface chemical changes, extends even into the case of core/shell structures. Accordingly, the goal of a more complete description of quantum dot surface chemistry has been a driver of innovation in colloidal nanocrystal purification methods. We specifically examine the effect of purification on surface chemistry and photoluminescence in quantum dots as an example of the challenges associated with nanocrystal purification and how improved understanding can result from increasingly precise techniques, and associated surface-sensitive analytical methods.

Received 3rd October 2016,
Accepted 2nd December 2016

DOI: 10.1039/c6cc07998a

www.rsc.org/chemcomm

1. Introduction

Nanocrystals (NCs) in many forms and compositions including metals, semiconductors, and metal oxides have been extensively studied in the past several decades. Numerous studies have shown that these emerging materials can be used in a wide range of applications from imaging and display to catalysis and solar cells.[1–7] In order to better understand the properties of NC samples and to speed the commercial development of these materials with confidence, recent studies have focused on precise control of the material structure including particle morphology, polydispersity and ligand population.[3,8–11] Synthetic procedures for the highest quality NCs frequently use an excess of surface-passivating ligands or surfactants to maintain the colloidal stability of the particles during growth and influence the NC morphology that is achieved. However, these excess ligands, as well as the byproducts generated by decomposition of reagent compounds remain in the crude product solution. Additionally, the solvent that is selected for synthesis is rarely the same as the medium into which the NCs will be placed for physical property measurements or applications. Accordingly, effective means for isolating nanomaterials from solution-phase mixtures are required. The most common purification procedure for nanoparticles, especially quantum dots (QDs) entails precipitation and redissolution (the PR method). In this procedure, addition of a miscible solvent in which the nanoparticles are poorly soluble (an anti-solvent) causes flocculation. The resulting flocculated nanoparticles precipitate rapidly (frequently accelerated by centrifugation), and can then be redissolved in a fresh solvent.

A challenge for any colloidal NC purification approach is that surface ligands can be subject to dynamic exchange between NC-bound and free-solution forms on experimental timescales; as a result, the actual surface may be a function of both the amounts and types of potential ligands that are present, and the solvent.[4,12–19] At the same time, residual impurities can affect

[a] *Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA. E-mail: greytak@sc.edu*
[b] *USC Nanocenter, University of South Carolina, Columbia, SC 29208, USA*
† Present address: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
‡ These authors contributed equally to this work.

shell growth and ligand exchange steps that are often required for NC end uses.[20,21] An effective means of isolating NC samples with well-defined properties is thus an important requirement for controlled NC synthesis and surface chemistry. While convenient, purification by PR entails several limitations that have been noted in studies of QDs.[22–24] As a result, there is increasing interest in alternative purification techniques, and some established techniques have shown performance that is comparable to or better than the PR method.

In this Article, we will review the current status of NC purification. Previous reviews have examined some of the methods described, especially for separating or refining samples composed of several types of nanoparticles.[25,26] In a few cases, a unique physical response of the material can be used for purification, such as purification of iron oxide nanoparticles by differential magnetic catch and release.[26] More generally, common purification methods can be grouped based on the fundamental physical property by which separation is achieved: namely polarity-based techniques, electrophoresis-based techniques, and size-based techniques. These techniques are generally applicable to a wide variety of nanoparticles including inorganic semiconductors, metals, metal oxides, and carbon dots. Recently, increasing attention has been brought to the role of the surface in controlling photoluminescence (PL),[15,18,27–30] redox potentials,[31] and carrier trapping[32] in QDs. Additionally, current priorities in the field are to establish a quantitative description of ligand binding and exchange,[16,22,29,33–35] which requires well-defined initial and final states; and to extend the success with "classic" materials such as binary chalcogenides to new compositions, including those that are immune to the health and environmental sensitivity of Pb and Cd.[1,36–38] It will be important to consider the surface chemistry of these new compositions as well. Here, we will emphasize the separation of colloidal NCs from small molecule impurities, with a focus on QDs and specifically the effect of purification on QD surface chemistry and PL, which is discussed in the last section.

2. Polarity-based purification techniques

2.1 Precipitation and redissolution

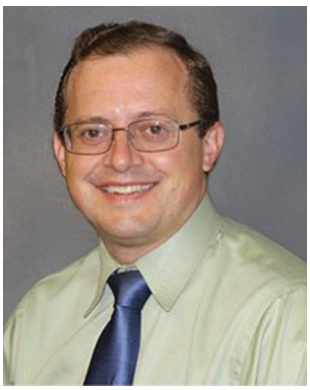
Since the development of QD syntheses in either aqueous solution[39] or high-boiling anhydrous solvents,[40] a precipitation and redissolution (PR) method has been described together with the preparation procedure, and therefore it is the first reported purification method for QDs. For the frequent case of QDs prepared in non-polar solvents, flocculation of QDs is achieved by introducing anti-solvents that increase the polarity of the solvent mixture. The supernatant, which retains impurities and excess ligands, can be decanted away and the QD precipitates can be redissolved in clean solvents. This process can be repeated several times until a desired purity of the NCs has been achieved. In addition to purification from synthetic solvents and byproducts, the PR method has also been used to refine the size distribution of the samples.[37,40–42] The particles are not fully precipitated

Yi Shen

Yi Shen received his BS in Chemical Engineering at Nanjing University, and completed PhD studies in Chemistry with the Greytak group at the University of South Carolina in 2015. A recipient of the SPARC graduate fellowship and the Dean's Dissertation Award at USC, he focused on QD purification, surface chemistry, and bio-imaging with polymer-coated QDs. He is presently pursuing postdoctoral studies in the Department of Chemical Engineering at MIT, in the laboratory of Prof. Klavs F. Jensen.

Megan Y. Gee

Megan Gee received her BS in Chemistry and Spanish from the University of South Carolina, where she continues as a PhD Candidate with the Greytak group. She is the recipient of USC's SPARC graduate fellowship and is currently focusing on measurement of nanocrystal–ligand interactions.



A. B. Greytak

Andrew Greytak received his BS in chemistry at the Massachusetts Institute of Technology in 2000. After completing his PhD at Harvard University under the supervision of Prof. Charles M. Lieber, he returned to MIT for a Life Sciences Research Foundation postdoctoral fellowship with Profs. Daniel G. Nocera and Moungi G. Bawendi. At the University of South Carolina, the Greytak group studies semiconductor nanocrystals, including measurements of nanocrystal ligand exchange and shell growth reactions that are pertinent to the development of highly capable nanocrystal fluorophores for bio-imaging applications, and to assembly of optoelectronic devices.

(crashed out), but rather the anti-solvents are titrated only until the particles just start to flocculate. It is frequently observed that the larger particles precipitate more readily than the smaller ones as the solvent polarity is increased, and on this basis they can be separated. The PR method is convenient and scalable, and has been utilized as the main purification technique in the majority of QD investigations. However, a number of studies have shown that there are some drawbacks to the PR method.

One of the main limitations is that the impurities and excess ligands sometimes have solubility properties similar to the nanoparticles, which makes them difficult to separate by the PR method. Many studies focusing on ligand chemistry describe multiple cycles of PR to remove free (unbound) and weakly adsorbed material. In some cases, even with a series of washing steps, the excess ligands cannot be removed by the PR method. For example, Nozik's group showed that the excess indium precursor cannot be removed from InAs QDs after using toluene/methanol to dissolve/precipitate QDs.[43] Chaudret and coworkers were able to eliminate a synthetic byproduct from InP QDs after identifying an appropriate PR system, but even after multiple washes could not isolate the NC product from residual octadecene solvent isomers interacting with surface coordinated carboxylate ligands.[44,45] Weiss's group has shown that impurities such as cadmium phosphonate and amides formed as precursor byproducts cannot be separated from CdSe QDs with chloroform/methanol as the solvent/anti-solvent for the purification.[46,47] Another severe issue is that the PR method may cause irreversible damage to the QD surface. One common observation is that the precipitated particles have a propensity to irreversibly aggregate after a number of necessary PR cycles, and so cannot be redispersed in solution.[48,49] Recently, a series of studies have shown that even for samples that maintain their colloidal stability, an improper choice of anti-solvent may remove ligands from the NC surface that were strongly bound in the original solvent. Owen's, Hens's, Weiss's and Meulenberg's groups have shown that methanol can react with the QD surface and displace bound metal carboxylate ligands.[22–24,50] This displacement results in adverse changes to the brightness of the samples, which will be discussed in greater detail below. Up to the present time, the PR method remains the most popular nanoparticle purification technique. However, the strong dependence of NC properties on the surface ligand population has also motivated researchers to study alternative purification techniques.

2.2 Extraction

An alternative method to separate NCs from the impurities, while still operating on the basis of differences in impurity and NC polarity, is extraction. The most common extraction system applied to QDs is liquid–liquid extraction. In general, the as-synthesized QD solution is stirred with an extracting solvent that is immiscible with the original solvent. By imposing a difference in the relative solubilities (partition coefficient) between the two liquids, the impurities and the QDs can be distributed into the different phases. In contrast with the popular PR method, the extraction method is intended to be a much gentler process, especially because the NCs tend to remain in their original phase during the purification, reducing the likelihood of irreversible aggregation or coalescence. However, numerous extraction cycles are commonly required in order to effectively remove a majority of the impurities because of the finite partition coefficients that can be achieved.

The most commonly used extraction system for typical colloidal QD systems is comprised of methanol/alkane, as the QDs are prepared in alkanes, and methanol (as the immiscible polar anti-solvent) is used to remove the impurities. Both Peng's group and Nie's group have applied this technique in purifying CdS, CdSe and CdTe based materials.[51–53] A limitation with this and similar systems, as shown in a recent study by Peng's group, is that when the metal precursor for the Cd-based synthesis is cadmium stearate, batchwise single stage extraction cannot effectively remove the impurities due to similar polarities between the particles and impurities.[54] To circumvent this issue, higher temperature and/or addition of co-extractants such as amines and phosphines were shown to significantly improve the extraction efficiency, as illustrated in Fig. 1.[54–56] Such nucleophiles are known to coordinate both metal carboxylate precursors and NC surfaces, and are commonly used in QD synthesis.[22] However, addition of such ligands during the extraction introduces an extra constituent to the purification process that could alter the final surface chemistry of the nanoparticles. The choice of both original and extracting solvents is important to the final extraction efficiency. Peng's group has shown that when the NC stock solution was extracted with a chloroform/methanol mixture, the impurities could be more effectively removed compared to the sample extracted with methanol only, each with hexane as the NC solvent.[53,54] Moreover, PR steps sometimes were used together with a sequence of several extraction cycles in order to completely segregate the nanoparticles from the impurities.[19,52,57]

The extracting solvent is normally chosen to have a higher solubility for the impurities, and negligible solubility for the QDs. Conversely, Jiang's group described using Triton X-114, a non-ionic surfactant, to perform cloud point extraction (CPE), which effectively concentrated and separated NCs from their original solution.[58] In this work, Triton X-114 formed a micelle to encapsulate the NCs and subsequently extract them when the temperature was above the cloud point temperature (CPT). The NCs could then be redispersed into an aqueous solution once the temperature was lowered beyond that CPT. This method provides an alternative way to concentrate the NCs, but it may prove difficult for NCs to be ultimately isolated from the added micelle-forming surfactants. Another interesting study was done by Pal's group, where they used copper stearate as a solid extracting agent to selectively remove amine ligands from gold organosol.[59] Similar to what Peng has proposed in the co-extractants study, the coordination effect between the amine ligands and metal carboxylate is the likely driving force for this separation.

3. Electrophoresis-based techniques

As the mobilities of nanoparticles and associated impurities under an electric field are generally different, electrophoresis

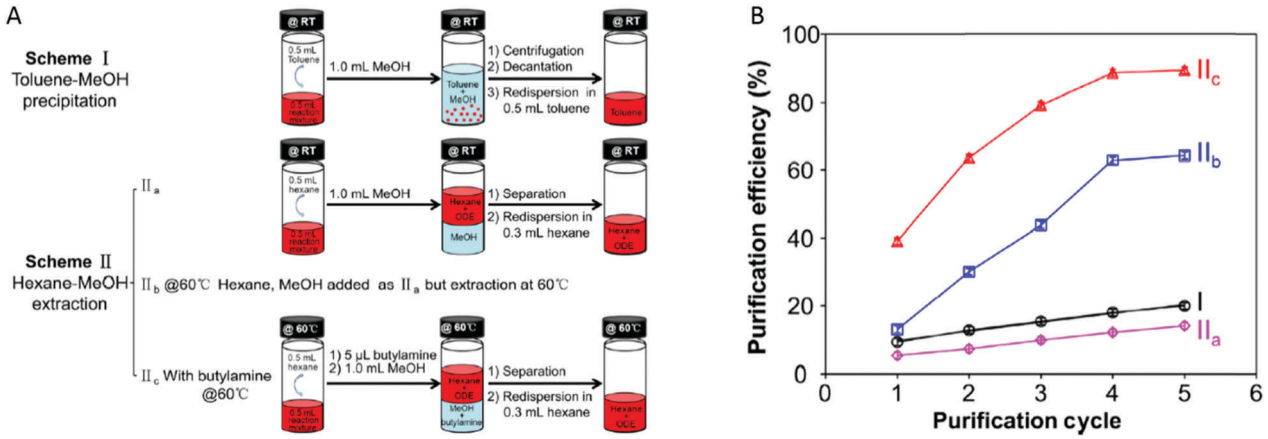


Fig. 1 (A) Schematic diagram of liquid–liquid extraction purification of stearate coated CdSe QDs examined in ref. 54. Only one cycle of purification is illustrated. (B) The purification efficiency of different methods described in (A). The purification efficiency is calculated by dividing the removed amount of cadmium stearate by the original amount of cadmium stearate in the QD solution. Reproduced with permission, Copyright Tsinghua University Press and Springer-Verlag Berlin Heidelberg 2015.

has been applied as another purification technique. In an early example, Claridge *et al.* used gel electrophoresis to separate Au nanoparticles with varying numbers of thiolated DNA strands adsorbed to the surface.[60] Later, Parak's group demonstrated that this technique could be used to separate polymer-coated CdSe/ZnS or Au nanoparticles with different functional groups;[61] the resolution was sufficient to distinguish particles specifically bearing zero to three desired conjugates. Electrophoresis has also been used to separate QDs bearing a single copy of poly-histidine tagged protein from unfunctionalized or multifunctionalized QDs.[62,63] Sonnichsen's group and Girault's group further showed that gel electrophoresis could be used to separate different shapes and sizes of Au and Ag nanoparticles functionalized with charged polymers.[64,65] However, as described in the work done by Kotov's group on CdTe nanoparticles, the gel-based process is extremely time-consuming, which is not ideal for applying this technique in continuous and large-scale separations.[66] Consequently, they described using free-flow electrophoresis (FFE), specifically to narrow the size distribution of CdTe nanoparticles. Separation *via* FFE was achieved by applying a high voltage electric field perpendicular to the direction of laminar flow, and differentiating based on the deflection of the charged particles. Employing this method, Kotov and co-authors were able to separate CdTe nanoparticles into more monodispersed populations on a preparative scale.

Electrophoresis generally requires aqueous systems, which would be a limitation for NPs synthesized by the typical organometallic route.[67] Recently, however, Dubertret's group has succeeded in implementing electrophoretic deposition (EPD) in nonpolar or slightly polar solvents as a means to sort Cd chalcogenide nanomaterials,[68] enabled by non-zero zeta potentials. In addition to employing electrophoresis as a size- or shape-selective technique, it has also been established for purifying nanocrystals of synthetic byproduct and excess surfactant impurities. Bass and co-workers described implementing EPD to purify metal chalcogenide nanomaterials containing a common surfactant layer of greasy organic ligands directly from their post-synthetic solution.[69] As shown in Fig. 2, by using reversible EPD processes, the nanoparticles can be effectively separated from the surfactant impurities in the suspension. After washing with polar non-solvents, the nanoparticles could be redispersed into clean nonpolar solvents. This method exhibited effective purification with no damage to nanoparticles, as confirmed by UV-Vis and NMR. The authors further demonstrated that this technique was versatile toward materials with different morphologies and capping ligands. Similar to the previously described PR method, however, the nanoparticles were removed from the solution phase during the purification and the particles had to be washed by the anti-solvents. Nevertheless, this electro-purification technique is more solvent-efficient and scalable compared to the PR method, which is an advantage from the perspective of industrial purification.

Jeong's group has attempted to develop the FFE method to purify CdSe QDs continuously on a microfluidic chip.[70] As shown in Fig. 3, the purified QDs could be separated from the unreacted precursors and excess surfactants by exploiting the electrophoretic movement of the particles. As one of the first forays into the continuous purification of nanocrystals, the yield and purification efficiency are not yet as good as what has been achieved in batch processes. Even so, this study exemplifies the progress that may be achievable through additional work on purification of nanoparticles in flow, in order to achieve continuous production of nanoparticles on a fully automated system.

4. Size-based separation

4.1 Ultracentrifugation

The significant size difference between the QDs and the impurities makes size-based separation an attractive alternative for NC purification. Ultracentrifugation, including density gradient centrifugation, is one of the most important size-based separation and characterization techniques in biological/colloidal studies. In contrast to the previously described PR method, in which the

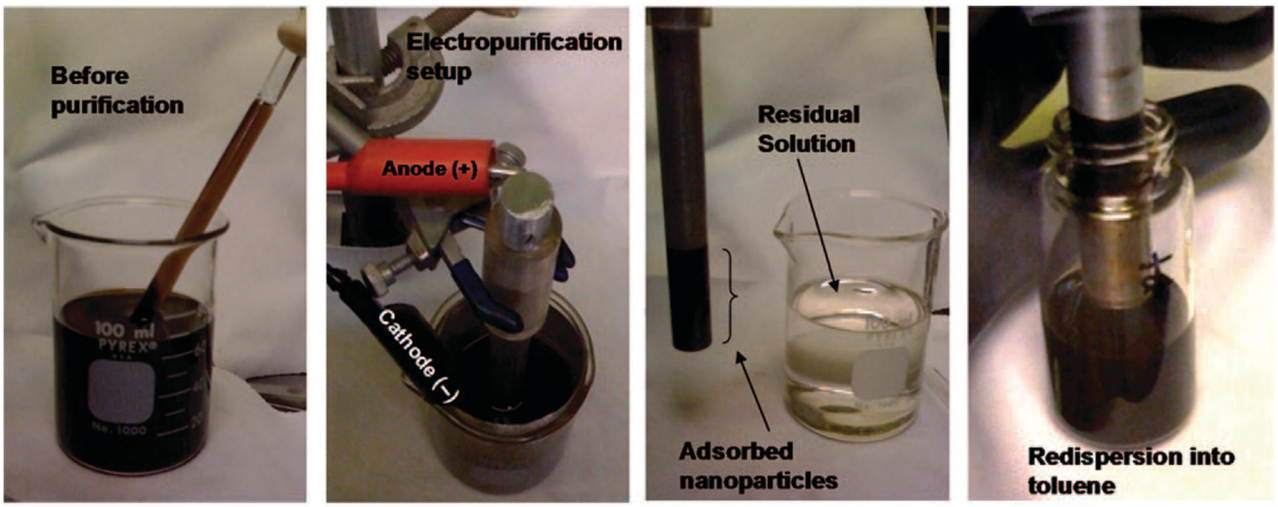


Fig. 2 Electropurification of as-synthesized CdTe nanocrystals described in ref. 69. Reproduced with permission. Copyright 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

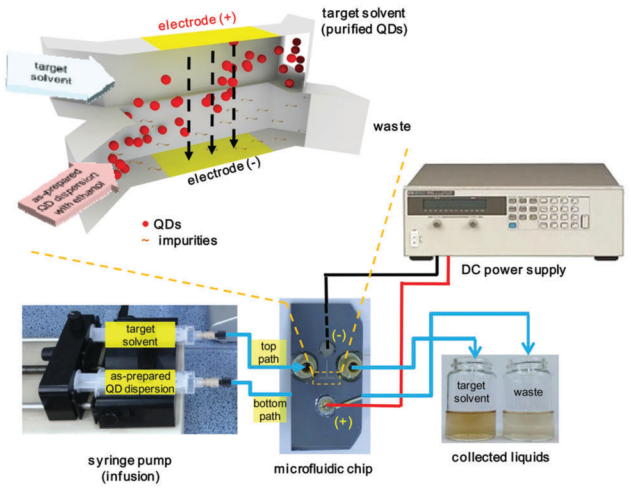


Fig. 3 Quantum dot electrophoretic purification setup described in ref. 70. Reproduced with permission. Copyright 2014 Royal Society of Chemistry.

introduction of anti-solvents causes nanoparticles to lose their solubility and flocculate into aggregates that can be sedimented with modest centrifugal force, separation *via* ultracentrifugation is achieved by the difference in sedimentation velocities among dispersed solutes. If the solute–solute interaction and diffusive effects are neglected, there are three main forces acting on a solute particle; namely centrifugal force, buoyant force and frictional force. The condition at steady state, when the above three forces are balanced, sets the Svedberg coefficient, defined as the sedimentation velocity normalized by the applied angular acceleration, equal to

$$S = \frac{v_s}{\omega^2 r} = \frac{V\left(\rho_p - \rho_l\right)}{f},$$

where v_s is the particle sedimentation velocity, ω is the angular velocity, r is the distance between particle and rotation axis, V is the particle volume, ρ_p and ρ_l are the density of the particle and solvent respectively, and f is the friction coefficient.[71] When the Svedberg coefficients of the solutes are different, separation can be achieved with centrifugal techniques.

Since the Svedberg coefficient depends linearly on volume, in principle size separation should be effectively achieved with ultracentrifugation. As reported in various articles, this technique has been used to characterize and reduce the size distribution of nanomaterials including single-walled carbon nanotubes (SWNTs),[72–74] metallic nanoparticles (MNPs),[75–80] semiconductor nanoparticles,[73,76,81–83] and oxide nanoparticles.[76] Moreover, when the size of nanoparticle is smaller than 20 nm in diameter, the ligands on the surface contribute substantially to the overall particle density, which further allows ultracentrifugation to separate nanoparticles with different coatings.[76,82,84] Ultracentrifugation can also be used to effectively sort nanoparticles with different shapes.[85–88] Recently, a mathematical model has been established by Sun's group for parameter optimization using ultracentrifugation for separation of polydisperse colloidal nanoparticles.[89]

As alluded to above, not only is ultracentrifugation convenient for preparing monodispersed nanoparticle samples, but it can also be used to isolate nanoparticles from excess functional ligands due to the significant size, density and friction coefficient differences between the particles and ligands. This method has frequently been used for the purification of QDs after surface modification for water stabilization. For example, Nie's group has used ultracentrifugation to separate excess polymer after NC encapsulation;[19] and Dubertret's group and Scholler' group have used ultracentrifugation to isolate NCs from phospholipid in a similar manner.[90,91] While most applications of ultracentrifugation have employed aqueous density gradients, which presents a general challenge of aggregation and instability for NCs synthesized *via* organo-metallic routes, investigations successfully employing this technique have been reviewed recently by Medintz's group.[92] Furthermore, Bai *et al.* showed that

ultracentrifugation can be used to purify as-synthesized nanomaterials in organic solvent from excess ligands such as oleylamine.[93] They obtained clear TEM images of metallic/semiconductor nanoparticles without any additional purification after size/shape separation was achieved. However, to the best of our knowledge, detailed surface characterization of the nanomaterials in organic solvents purified by ultracentrifugation has not been performed, nor has its efficiency as a purification technique been compared with other methods.

4.2 Membrane-based separation

Separation using a membrane prepared by porous materials is another attractive method to narrow the size distribution of the as-synthesized nanomaterials, as well as isolate the nanomaterials from the excess ligands. Typically, dialysis and filtration are the two general techniques with size-based membrane separation involved. In the process of dialysis, a membrane should be selected with an effective molecular weight cut-off (MWCO) that permits the excess ligands and other small molecule impurities to diffuse from the high concentration area (sample solution) to the low concentration solution (dialysate solution) until equilibrium is reached, but the pores must of course be small enough to prevent nanoparticles from transiting. After repeating the dialysis process several times, the concentration of the excess ligands in the sample solution will significantly decrease while the concentration of the nanoparticles can be maintained. This method has frequently been used for the purification of nanoparticles in aqueous solution, and is generally done using commercially available membranes (dialysis tubing). For example, Stone's group and Ren's group have used dialysis to purify as-synthesized Au and CdTe nanoparticles in water;[94,95] Snee's group has recently used dialysis as a gentle technique to purify the water soluble QDs after silane coating;[96] and dialysis has also been used to isolate bio-labeled nanoparticles from excess bio-linkers after the functionalization reactions.[96,97] One drawback of dialysis is that this process is time consuming as it normally takes several hours or more to allow the equilibrium between concentrations of small molecules at the two sides of the membrane to be reached.

Distinct from dialysis, where the separation is achieved by diffusion of the small molecules, filtration is more efficient in time, exploiting an extra driving force in the form of a difference in fluid pressure to accelerate the separation. The challenges lie in selecting a filter membrane with an appropriate size cutoff and solvent tolerance, and avoiding aggregation of the nanoparticles as they accumulate at the membrane. A wide variety of filtration systems has been developed to purify and retain biomolecules such as proteins in aqueous solvents, typically using centrifugal force or vacuum to generate the pressure difference. Accordingly, centrifugal filtration has in many cases replaced dialysis for purification, buffer exchange, and concentration of water-soluble biomolecules and nanoparticles, especially those that will not aggregate during the filtration process. QDs stabilized in aqueous solution by ligand exchange or surfactant encapsulation are often purified by this method.[8,98,99] Snee *et al.* used centrifugal filtration to purify pH sensitive dye-conjugated CdSe/ZnS QDs to develop a ratiometric pH sensor.[100] Though commercial membranes were primarily designed to retain proteins and synthetic colloidal particles while passing only small molecules, researchers have developed membranes with larger size cutoffs that can be used to separate small particles from larger ones to refine the size distribution of the sample – a nanoscale form of sieving. Mesoporous silica hybrid membranes have been used to perform size separation of metal nanoparticles prepared in either aqueous or organic solvents.[101–103] Rybtchinski's group has also described using polymer based membranes to perform size separation on metal nanoparticles and QDs in aqueous solution, as the organic solvent may lead to disassembly of the supramolecular structure.[104]

Ultrafiltration and diafiltration are two additional membrane-based separation technologies for nanoparticle purification. Ultrafiltration is also known as tangential flow filtration (TFF), and operates such that the feed solution flows parallel to the membrane surface, instead of perpendicular to it as in conventional ''dead-end'' filtration. A concentrated nanoparticle solution is retained. Many commercial centrifugal filtration units actually accomplish TFF, but ultrafiltration can also be scaled up for industrial process sizes. The set-up and operating principle of diafiltration is very similar to ultrafiltration; the only difference being that extra solvent is injected into the sample solution to maintain a constant total volume of the feed during the purification process. One major advantage of both ultrafiltration and diafiltration is that these two processes can be operated semi-continuously for in-line nanoparticle purification. Ulbricht's group has applied ultrafiltration and diafiltration to purify gold and silica nanoparticles from excess small ligands or biomolecules.[105,106] Hutchison's group has performed a systematic study to compare the purification efficiency of diafiltration with other techniques on thiol-PEG coated Au nanoparticle samples.[107] As shown in Fig. 4, all of the sharp peaks in the ^{1}H NMR spectrum within the 2.0–4.5 ppm region have been removed by diafiltration, whereas these free ligand features remained in the samples purified by other methods. Their results demonstrated that diafiltration is an efficient purification technique for nanoparticles in aqueous solution. Similar to ultracentrifugation, most of the studies utilizing membrane-based separation still focus on the nanoparticle samples in aqueous solution. In order to purify nanoparticles prepared in organic solvents using membrane separation, advances in membrane technology will be welcomed.

4.3 Chromatography

Chromatography is another general strategy by which nanoparticles can be effectively purified, and one that the authors have pursued for colloidal QDs. Whether preparative or analytical in scale, the premise of chromatography involves the elution of mixtures dissolved in a mobile phase (MP) through a structured stationary phase (SP), between which the sample components are partitioned. In affinity chromatographies, some specific and/or enthalpic interaction between the sample components and the stationary phase determines this equilibrium. Affinity chromatography has been developed to effectively isolate bio-conjugated nanoparticles from excess unbound peptides and unfunctionalized

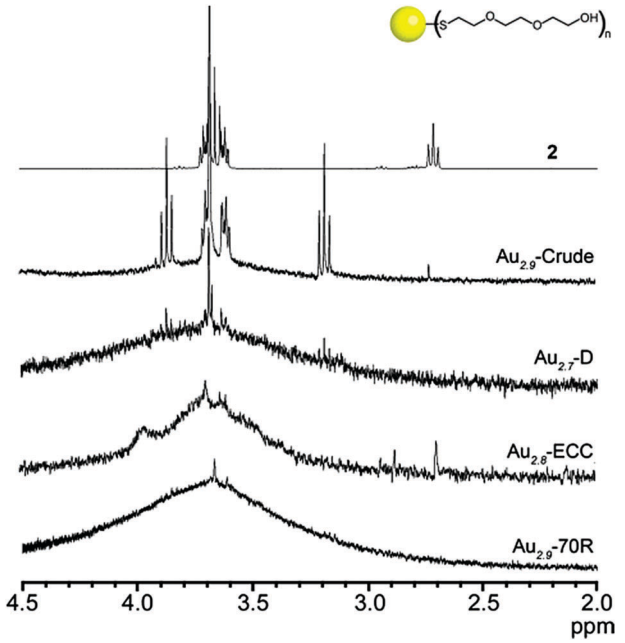


Fig. 4 Proton NMR spectra of thiol-capped Au nanoparticles purified by several methods. From top to bottom: the pure oligo(ethylene glycol) ligand (marked **2**), as-synthesized Au nanoparticles ($Au_{2.9}$-crude), dialyzed nanoparticles ($Au_{2.7}$-D), nanoparticles purified *via* extraction, chromatography, and centrifugation ($Au_{2.8}$-ECC), and nanoparticles purified *via* diafiltration ($Au_{2.9}$-70R). The absence of the sharp peaks in the diafiltered sample indicates the removal of the free ligands. Reproduced with permission from ref. 107. Copyright 2006 American Chemical Society.

peptide-bearing nanoparticles.[60,108,109] Alternatively, purely physical (entropic) interactions can be used to separate components on the basis of size. Because of the large variation in size among components in colloidal nanoparticle samples, the latter approach holds considerable appeal. High resolution in separations and resulting chromatographs (often times coupled with additional detection equipment, *e.g.* absorption, photoluminescence, and mass spectrometers) provides *in situ* characterization of analytes in terms of size, surfactant interaction, states of thermodynamic stability and equilibrium, and purification.

Among inorganic materials, Au NPs have the most extensive chromatographic application history,[67,79,109–118] and as such have offered an important framework to develop such methods applied to characterize and purify QD systems. Tiede *et al.* developed hydrodynamic chromatography (HDC) coupled with ICP-MS for Au NPs as well as a number of oxide NPs,[110] and Pergantis *et al.* improved HDC detection limits for the study of Au NPs in environmental matrices.[111] HDC utilizes a nonporous SP, and the mechanism for separation is based on the parabolic flow profile of a Newtonian fluid in an open tube or within the interstitial volume of the column, such that smaller particles can migrate towards the outer areas while larger particles are deflected towards the center where the MP flow rate is fastest.[119] The most popular and versatile chromatographic technique applied to NP suspensions, however, is size exclusion chromatography (SEC).

The general principle by which SEC operates is that analytes fractionate between the mobile phase and a porous stationary phase to an extent that depends monotonically on their hydrodynamic size, with larger particles eluting more rapidly than smaller constituents that can traverse the pores of the SP. The stationary phase is typically made up of cross-linked polymer gel beads characterized by an effective molecular weight cutoff (MWCO) above which analytes are largely excluded from the pores and transit only the ''void volume'' between gel beads. SEC can be accomplished with polar mobile phases (including water) using hydrophilic polysaccharide or polyacrylamide gels; this represents one form of gel filtration chromatography (GFC), a term that also encompasses affinity chromatography methods using functionalized hydrophilic gels. SEC with anhydrous mobile phases is termed gel permeation chromatography (GPC) and is routinely used to characterize polymer molecular weight distributions. Innovation in high-performance liquid chromatography (HPLC) technology has led to sophisticated instrumentation for both GFC and GPC. In its more conventional use, application of SEC to nanotechnology has sought to improve NP sample polydispersity through higher resolution sensitivity for both size and shape.[1,2,25,79,113,120–129] Differences in structure (hard spheres *versus* folded proteins or randomly coiled polymers) require that columns be properly calibrated against nanocluster standards[123] or polystyrene;[128] nevertheless, most investigations have successfully determined size-exclusion fractionation mechanisms.[117,130] One of the major challenges in this technique, which distorts elution profiles in QDs and other NPs alike, is the possibility of enthalpic interactions that result in increased retention or irreversible sorption of the sample onto the stationary phase. This makes it imperative to identify appropriate SP media. The most common strategy employed to circumvent this critical issue is to include MP additives that may include surfactants,[25,79,122–125,131,132] but a number of recent reports[20,49,116,118,133–137] have also demonstrated that suitable SP properties have been achieved with both polar and nonpolar MP solvents. Fig. 5 demonstrates early success in resolution capacity when a properly chosen SP is combined with multiple on-line detectors for SEC utilized as a high pressure liquid chromatograph for nanoparticle separation. A cross-linked polystyrene SP, with a relatively larger pore size than was used when only eluting Au clusters in the same investigation, was combined with tetrahydrofuran as the MP.[123]

An early application of HPLC/SEC to QDs was successful in resolving elution fractions that contained narrower size distributions than the starting material. However, recent studies have shown that this technique could also be used to purify nanocrystals. The potential for SEC to achieve size separation as well as QD purification is apparent in Fig. 5 as 2.0 nm Au nanoclusters and 1.8 nm CdSe NCs are well-resolved from linear alkanes that could conceivably represent excess ligand or surfactant impurities. Early examples to affect a type of purification with HPLC/SEC applied to QDs were mainly applied post-surface modification to separate QDs from excess polymers.[130,132,138] Shortly thereafter, Biesta *et al.* utilized GPC in a similar manner to separate excess dye molecules from nanocrystalline silica in an organic MP.[139] In a series of investigations toward biocompatible surface modification, water-soluble core/shell QDs

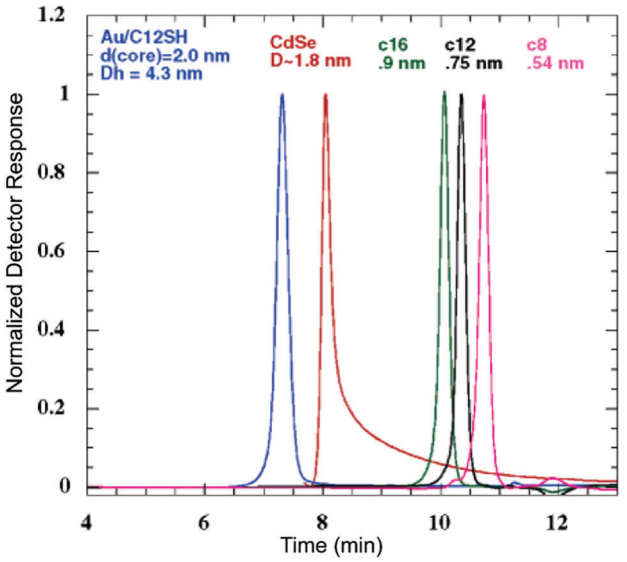


Fig. 5 Chromatograms of normalized detector signals as a function of elution time using 1000 Å cross linked polystyrene SP with THF MP for d = 2.0 nm alkanethiol-capped Au clusters (blue), d = 1.8 nm pyridine-functionalized CdSe NCs (red), hexadecane (c16, green), dodecane (c12, black) and octane (c8, pink). Retention times are well resolved and demonstrate SEC separation mechanism. Adapted with permission from ref. 123. Copyright 2005 American Chemical Society.

functionalized with hydrophilic ligands were successfully analyzed with aqueous gel filtration chromatography (GFC) on the basis of their resultant sizes; this was used to demonstrate the strength of ligand binding and to assess the presence or absence of non-specific adsorption of highly prevalent, endogenous serum proteins in QDs intended for bioimaging applications.[63,140–142] In a preparative example, McLaurin *et al.* used GFC to isolate QD-based ratiometric oxygen sensors.[143] QDs functionalized with an oxygen-sensitive, osmium-based FRET acceptor phosphors were separated from QD aggregates and unbound phosphors, as a step toward QD FRET-based oxygen sensors applicable in biological systems. A very recent investigation of Cd-based QD bioprobes purified by HPSEC post-ligand exchange confirmed significantly improved agglomerate removal, and therefore negligible nonspecific binding, with enhanced performance of the QD bioprobes in immunofluorescence imaging and stability for at least one year.[135]

Our group has recently established and continues to develop gel permeation chromatography (GPC) as a preparative, reproducible and robust purification technique for colloidal NCs (Fig. 6).[20,116] This novel approach for NC purification utilizes GPC to exert a continuous driving force *in situ* to separate unbound or weakly-bound small molecules. Only those ligands with very large association equilibrium constants and/or very slow desorption kinetics will remain adsorbed to the NC surface upon their elution from the column. A MP solvent can be selected that is the same as, or of similar polarity to, the initial solvent for the NCs and so maintains the solvent conditions under which the initial surface coordination environment was assembled.[20,29] The SP consists of cross-linked polystyrene (Bio-Rad's Bio-Beads SX-1 in initial studies). This SP has proven to be compatible with Cd-based core and core/shell QDs capped with typical anionic and L-type ligands, polymer-functionalized Cd-based QDs with polar organic MP,[116,132] carboxylate-capped InP QDs,[116,144] thiol-capped Au NCs,[116] Cd-based nanorods of varying aspect ratios,[116] perovskite NCs,[145] and iron oxide NCs.[133,134] Moreover, it is also possible to use the GPC column as a multi-functional flow reactor that can accomplish in sequence the steps of initial purification, ligand exchange, and subsequent cleanup without requiring a change of phase. The dearth of SP media for preparative SEC that swell in organic solvents remains a limitation for the use of SEC for NP purification, given the relatively low MWCO and operating pressures of polystyrene Bio-Beads.[24,121,132] Technological emphasis in resolution and operating power has instead focused on small sizes.[129] Aside from typical column packing material used in GFC or other aqueous phase HPLC/SEC, a few additional SP media with organic MP have been identified as suitable for NCs. Namely, a cross-linked styrene/divinyl benzene SP, which is both similar to and compatible with polystyrene, was effective in eluting Cd-based core/shell QDs.[128] It is anticipated that SP media can be identified, or specifically engineered, to meet the need of a higher MWCO for larger synthesized NPs and overall scalability. It will be essential to carefully consider and test the compatibility of SP media with the NCs and the impurities being removed, to prevent or minimize enthalpic interactions or aggregation on the column and achieve high resolution separations.

5. Influence of purification on photoluminescence in colloidal QDs

Purification steps can alter free ligand concentrations and the solvent, so the need to understand how NC surfaces are affected by purification has become more apparent as it is typically the first processing step towards applications.[3,4,14,15] Efficient and size-tunable photoluminescence is one of the most distinctive characteristics of colloidal QDs and has led to their initial commercial applications, and indeed the most facile and globally employed methods to monitor changes in QD electronic structure are band edge optical absorption and emission. Narrow, well-resolved, and consistent features in continuous-wave absorption spectra are sensitive to changes in QD radius and polydispersity, but reveal limited or no information about localized states. The PL quantum yield (QY) and lifetime are sensitive to trapping and non-radiative decay; in some cases radiative decay from trapped excitons can be observed. Time-resolved absorption and PL spectroscopy can additionally reveal reversible trapping, and information about the distribution of non-radiative decay rates that is present among QDs in the sample.[146] This section will present some of the changes in optical properties of QDs associated with purification as an example of how purification influences NC surface chemistry and electronic properties.

Purification methods can modify the QD surface through dissociation or exchange of various ligand types with a number

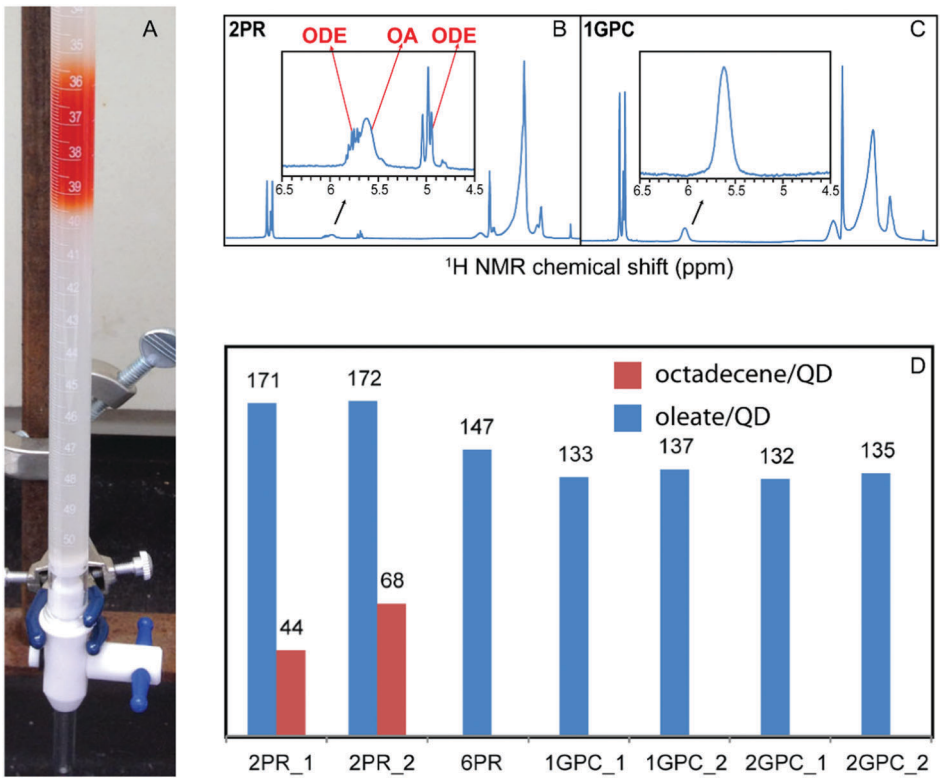


Fig. 6 GPC purification of oleate-capped CdSe QDs with toluene MP and polystyrene SP (A) photograph of QDs (red band) transiting column under gravity pressure. (B and C) ^{1}H NMR spectra of 2 cycle precipitation/redissolution purified (A) and GPC purified (B) CdSe QDs. The insets are the expanded views of the spectra in the range 4.5–6.5 ppm, capturing the olefin proton resonances of oleate species (OA) and residual octadecene synthesis solvent (ODE). (D) Ligand/impurities-to-QDs ratio (mole/mole) for CdSe QDs purified by different methods. Adapted with permission from ref. 20. Copyright 2013 American Chemical Society.

of possible binding modes. Therefore, it is important to understand how ligand binding affects PL, and whether PL changes observed on purification, dilution, or a change of solvent are a direct result of ligand exchange, or are associated with aggregation, surface oxidation, or surface reconstruction that may be irreversible.[23,27,37,50,147,148] The ligands and solvent also directly modulate energy levels through classical dielectric confinement effects, though this has been shown to be a small contribution in most cases.[149,150]

5.1 Surface coordination of compound semiconductor QDs

It is crucial to identify and conceptualize ligand binding modes in order to understand the major role purification can play in modulating QD electronic properties and chemical reactivity in subsequent processing steps.

Much of what has been discovered about as-synthesized QD–ligand binding dynamics has emerged in the course of surface modification procedures designed to replace native ligands.[151,152] Early understanding of QD growth hinged on the concept of a coordinating solvent: the use of compounds, typically Lewis bases, in high concentration to saturate the surfaces of particles and serve as a steric barrier to aggregation even at high growth temperatures.[40] The role of the coordinating solvent and identity of post-synthesis capping ligands have been challenged by evidence that strongly coordinating reagent impurities, rather than the nominal solvents themselves, can dominate precursor reactivity and can also become the most tightly bound and prevalent surface species, retained even through multiple purification cycles.[20,46,153]

Motivated by the need to clarify how interactions between native ligands and QD surfaces influence further surface modifications, particularly in ligand exchange reactions, Owen's group and Hens's group have employed the covalent bond classification (CBC) to characterize the exchangeable groups, initially based on Cd chalcogenide QDs.[3,22,151,154] In this scheme, ligands are recognized as either L-type (2-electron donors, dissociating to yield neutral Lewis bases); X-type (formal 1-electron donors, dissociating to yield ions); or Z-type neutral Lewis acids (2-electron acceptors, dissociating to yield neutral Lewis acids). The original coordinating solvents and neutral carboxylic acids can thus be described as L-type, while carboxylates or halides are X-type. The binding between L-type ligands and QDs can be labile especially when compared to strong-binding X-type ligands. L-type ligands can be subject to dynamic exchange between bound and free forms on experimental timescales. As a result, following le Chatelier's principle, this should lead to a predictable loss of such ligands in any of the purification techniques mentioned in the previous sections. Dissociation of X-type ligands in non-polar solvents will not occur easily as it requires charge separation, radical formation, or reductive elimination. Drift velocity measurements have revealed that QDs with X-type ligands in a non-polar solvent carry no more

than a few elementary charges.[155] However, as mentioned earlier, numerous studies revealed that purification by multiple PR cycles, especially with protic anti-solvents such as methanol, caused displacement of lattice-terminating core cations together with charge-balancing anionic ligands (Z-type ligands).[22,24,54,156,157] Therefore, in common purification practices, the ligands that have been removed are either L-type or Z-type, and the PL changes upon purification are predominately a result of changes in L-type or Z-type ligand population. The relative strength of L-type ligand binding appears to vary widely: phosphines and amines are labile at room temperature at CdSe and CdS NC surfaces, while primary amines exhibit very strong binding to $CuInS_2$ QD surfaces and were found to be labile only on heating.[158]

While the covalent bond classification as described above has served as a useful model for describing binary chalcogenide QDs coated with carboxylates, phosphonates, and amines, it is likely an incomplete picture of ligand binding modes. For example, thiols are well known as strong ligands for many metal and metal chalcogenide NCs. Within the CBC above, thiols could bind as L-type ligands *via* their lone pairs, or as X-type thiolates following deprotonation. However, the MacDonald group has reported[159,160] purification and ligand exchange results indicating a further distinction between "surface-bound" thiolates that are subject to exchange, and thiol-derived "crystal-bound" sulfur that retains a covalent bond to an organic tail group, but exists in a highly coordinated environment resembling the anion site of the crystal lattice. Crystal-bound thiol ligands on Cu_2S, $CuInS_2$, and CdSe/ZnS QDs are resistant to loss or displacement in purifications steps.

Purification techniques can be an important tool in studying and manipulating NC surface chemistry. Ligand binding and exchange can be studied *via* NMR and isothermal titration calorimetry (ITC) by introducing putative ligands to purified NCs. Additionally, purification methods that do not require a change in solvent offer the opportunity to directly characterize ligand dissociation kinetics and thermodynamics. For example, by continuously exposing NCs to a low or zero concentration of free ligands, only those surface-bound equivalents with off-rate lifetimes greater than the experimental timescale will remain. Purification at different speeds could potentially detect off-rate kinetics for molecules with very small dissociation equilibrium constants. This data could help to distinguish associative and dissociative ligand exchange mechanisms.[161] Ligand exchange rates could be investigated as a function of solvent polarity or the presence of solvent impurities such as moisture,[162] which has already been shown to play a role in NC growth kinetics but has not been thoroughly examined in the context of ligand exchange to our knowledge. Quantitative kinetic and thermodynamic measurements using purification may emerge in the near future, though a concern is the propensity of NCs to aggregate if steric and/or electrostatic stability imparted by labile surface groups is lost.

5.2 Associating PL changes with effects of purification

It is well known that ligand exchange can affect QY; for example, introduction of thiols has frequently been seen to quench QY in CdSe QDs and this has been attributed to the formation of hole traps at the QD surface.[4,18,163,164] However, changes have been observed upon mere purification or dilution as well. In 2006 Bullen and Mulvaney published a quantitative investigation on the relative strength of binding for ligands as a function of solvent polarity, purity or temperature;[18] it was one of several reports around the same time that demonstrated how merely diluting QD suspensions displaced chemisorbed ligands.[27,28] The PL response observed by Bullen and Mulvaney suggested an effective binding constant governs fractional occupation especially of L-type ligands at the QD surface. Indeed PL quenching on purification is seen in many cases.[30,165] Fortunately, it has now been established that such luminescence responses are frequently reversible, as demonstrated in both absorption and emission spectra.[27,29,54,166–168] Fig. 7 depicts this phenomenon in two separate investigations conducted 10 years apart, but attaining remarkably similar profiles. On the left Kalyuzhny and Murray observed a slight redshift of band-edge absorption and PL maxima of diluted CdSe QDs at the conclusion of a number of purification steps. Additionally, sub-bandgap emission attributed to surface defects increased while band edge emission decreased by almost 90% of its original intensity with repeated purification steps.[27] The reverse of this process is depicted in the later study conducted by Krause *et al.* on the right, with the addition of an L-type ligand (butylamine) capable of passivating surface cadmium sites, suppressing surface electron trap emission and increasing overall PL efficiency.[147]

Our group has investigated PL dynamics in core/shell Cd–chalcogenide QDs, which displayed a characteristic decrease in

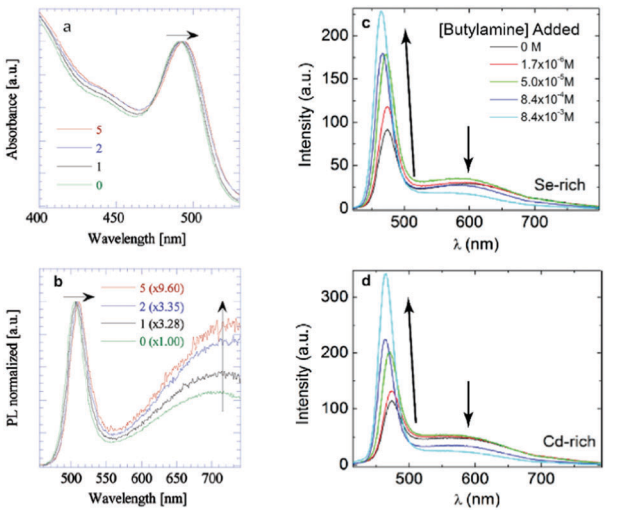


Fig. 7 Left: Normalized optical spectra for CdSe QDs showing redshift in (a) band-edge absorption and (b) emission upon 0, 1, 2 and 5 times repeated PR purification cycles recorded by Kalyuzhny and Murray in 2005 (ref. 27). Lower energy defect emission increases substantially with repeated PR as shown in (b). Right: Addition of butylamine at relevant concentrations after sufficient time for QD surface equilibration, demonstrating reversible band-edge and defect emission shifts for (c) Se-rich and (d) Cd-rich CdSe QDs recorded by Krause *et al.* in 2015 (ref. 147). Figures adapted with permission. Copyright American Chemical Society 2005 & 2015.

PLQY, decrease in average lifetime, and increase in rate dispersion when purified by GPC in toluene.[29] After each surfactant component from the shell growth reaction was systematically reintroduced to the purified core/shell QDs, it was observed that alkylamine and trioctylphosphine (TOP) L-type ligands could most effectively and reversibly recover QY (Fig. 8). The QY regeneration was accompanied by restoration of the PL lifetime; the lifetime changes were associated with a change in the amplitude of the longest lifetime components.

We employed isothermal titration calorimetry to differentiate the extent of L-type ligand binding for the thinner CdZnS alloy shell sample, which revealed an exotherm associated with introduction of TOP to purified QDs, confirming an interaction of the ligand with the QD surface, whereas trioctylphosphine oxide, which failed to restore the QY, did not generate a heat response, consistent with a lack of binding over the same range of concentrations.[29] A binary L- and X-type ligand system could universally regenerate QY for the purified QDs, however this was attributed to irreversible surface reconstruction as evidenced by bathochromic shifts in related optical spectra.[29,167,168] The change in PL upon GPC purification could thus be attributed to a complete loss of L-type ligands, while a population of strongly-bound Z-type ligands is retained on the experimental timescale. Akdas *et al.* conducted an investigation to elucidate effects of purification on optical properties in $CuInS_2$ and $CuInS_2$/ZnS which revealed very similar PL lifetime amplitude trends once an optimal purification system was identified.[38] Despite the roles of the thiol as both a NC precursor material and final passivating ligand, which should lead to a crystal-bound thiol coating, the core passivation alone did not make the opto-electronic properties impervious to purification. As with typical observations, the QY and average PL lifetime were better maintained when electronic isolation was provided by a ZnS shell, and even more so when this was combined with a judicious choice of the PR anti-solvent/solvent combination for their purification.[38] Another recent study developing an aqueous-based gradient ultracentrifugation purification for fluorescent carbon NPs, also reported that maintaining exciton radiative recombination was influenced primarily by retained hydrophilic surface groups.[169] While it would be inappropriate to consider all QD surfaces as completely analogous to Cd–chalcogenide systems, these observations suggest that at least some observations can be transferred to more complex semiconductor–ligand interfaces.

Over the last few years a number of groups have published reports on less toxic alternatives to the very familiar Cd and Pb chalcogenide NCs, and the emerging systems are usually more complex quantum-confined fluorophores.[1,38,154,169] Findings from Cossairt's group after a closer investigation of shell precursor material added to InP QDs to improve QY, revealed another example of NC surface engineering. When adding what they term "exogenous Lewis acids" in the form of specific Cd- and Zn-carboxylates, the QD surface was altered such that significant PL enhancement could be achieved,[36] as well as a tuning of the InP absorption and emission lowest energy electronic transitions. QY enhancement upon the post-synthetic treatment with the metal carboxylates might be described as passivation through introduction of a Z-type ligand. If so, the ligand association is very strong, as a portion of the added metal was retained even after excess metal carboxylates and In-oxide impurities were removed by a combined PR and filtration purification process. Final In-to-metal ratios along with the PL responses suggested that the metal carboxylates strongly adhered to undercoordinated surface phosphorus sites, and to some degree were also integrated in the crystal lattice to displace native In atoms. Again, while Cd-based NC investigations dominate the field and offer an important foundation for other complex semiconductor–ligand interfacial studies, the responses to purification in this metal-doped InP system cannot be directly interpreted on the basis of the Cd chalcogenide system; especially since the drastic changes in lowest energy electronic

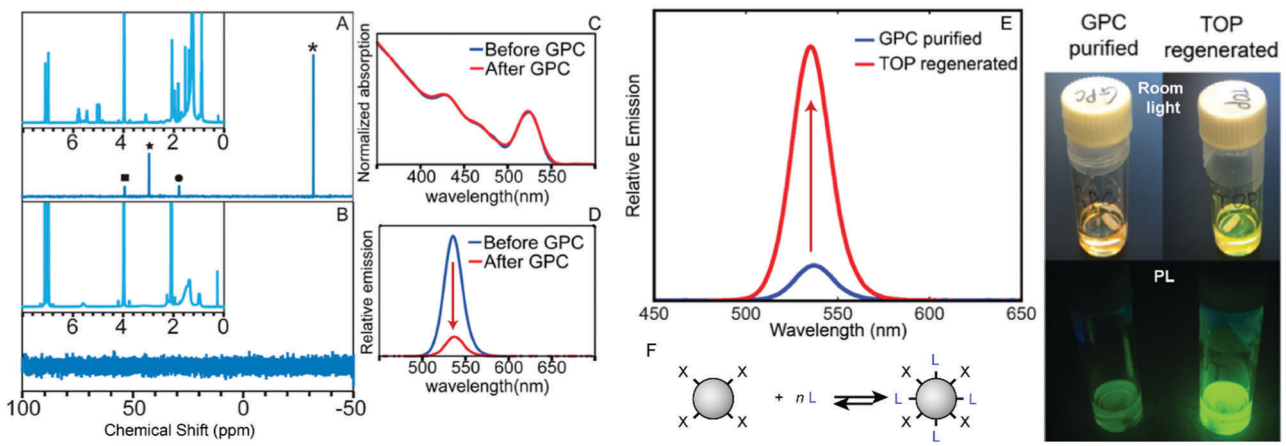


Fig. 8 ^{31}P NMR of core/shell CD-based QDs before (A) and after (B) GPC purification; insets of respective ^{1}H NMR spectra. (C) Absorption spectra of QDs demonstrating no surface damage after GPC purification. (D) Decrease in band-edge PL intensity after GPC purification, and (E) PL intensity regenerated after addition of L-type ligand post-purification. (F) Schematic of L-type ligand association to vacant sites on QD surface; only strongly coordinated X-type ligands remain post-purification. Photos of GPC-purified QDs before and after QY regeneration QDs under ambient light (top) and UV irradiation (bottom). Figures adapted with permission from ref. 29. Copyright 2015 American Chemical Society.

transition wavelengths were not accompanied by a change in the NC size. The ability to identify reversible or non-perturbing purification techniques tailored to specific QD materials in specific processing environments is imperative. This is especially true if analysis of the final product is difficult; for example if ligand exchange leads to a quenched product, it may be difficult to distinguish successful ligand exchange from repercussions of purification.[35]

5.3 Improving structural interpretation and material scope

Many descriptions of NC ligand binding modes are purely schematic and do not account for the specific structural characteristics of the NC surface. Yet, this is beginning to change with more examples of single-crystal X-ray diffraction based structures for inorganic clusters and small NCs,[10,170] and with more closely integrated theoretic and experimental analysis. The crystal phase, size, and stoichiometric excess of NCs influence the number and character of the ligand binding sites that are available at their surfaces. For example, the eight symmetrically equivalent {111} faces of the rocksalt crystal structure adopted by PbS NCs are formed by alternating layers of cations and anions; terminating them with a complete monolayer results in a charged surface requiring a high density of X-type ligands for charge balance, while the {100} and {110} facets are charge-neutral. This effect of this requirement was demonstrated in a combined theoretical and experimental study by Zherebetskyy *et al.*[171] of oleic acid-passivated PbS NCs, which showed that water generated as a synthetic byproduct results in hydroxide serving as an X-type ligand at the {111} facets. Hydroxide was necessary to completely passivate the {111} facets, in addition to oleate, due to steric constraints. In contrast, the {100} facets could be passivated by readily exchangeable oleic acid. An investigation on ligand interactions at certain CdS facets[34] further supported the increasing need to probe QD surface structure to control processes such as interparticle charge transfer or extended crystal growth.

Precise purification methods, supported by surface metrics that can gauge their effects, should enable the handling of NCs with sub-monolayer surface termination and accurate measurement of ligand stoichiometries. As such purification technologies will be a useful tool in developing structurally grounded models of surface chemistry and ligand exchange at NC surfaces. Purification technologies must also be shown to work for emerging classes of nanocrystalline materials, including Cd- and Pb-free compound semiconductors such as InP and LiZnP.[1,36,172] These compounds can have more complex ligand binding modes and increased sensitivity to oxidation compared to chalcogenides, which will increase the need for precision in describing their surface chemistry and spectroscopy.

6. Conclusions

As the value contributed by colloidal NCs becomes increasingly clear, the need for a detailed understanding of their surface chemistry and of appropriate means to describe and isolate the structures that give rise to their remarkable and useful properties becomes increasingly urgent. The work described in this Article clearly illustrates that the surfaces of colloidal NCs are subject to dynamic equilibrium with the surrounding medium and may change upon purification or dilution, leading to changes in properties critical to performance. Purification methods are now available that allow a high degree of process control and repeatability, and many of them do not require a change in solvent that could disrupt ligand binding free energies. Additionally, analytical techniques such as NMR, TGA, and calorimetry can help to profile samples to allow the results of different purification techniques to be compared, and in principle to permit a standard description of nanoparticle samples across the field.[173] Such standardization will greatly benefit interpretation of physical property measurements and accelerate the development of more sophisticated, multistep preparative routes to highly efficient absorbers, emitters, and multifunctional materials. Increased rigor in reporting of purification methods and analytical metrics will be helpful in achieving these goals. Ultimately, matters of scalability and cost will be important in selecting the most appropriate purification method for a particular task. Engineering studies examining purification of nanocrystals at larger scale and in integrated and/or continuous processes could help optimize current technologies and achieve useful cost figures.

Acknowledgements

The authors gratefully acknowledge the support of the US National Science Foundation under award #1613388 and of the University of South Carolina, including a SPARC Graduate Research Fellowship and Dean's Dissertation Fellowship to Y. S., and a SPARC Graduate Research Fellowship to M. Y. G. Additional support was provided through South Carolina EPSCOR grant #EPS-0903795 to A. B. G. (a sub-award from NSF grant #1317771).

References

1 P. Reiss, M. Carrière, C. Lincheneau, L. Vaure and S. Tamang, *Chem. Rev.*, 2016, **116**, 10731–10819.
2 E. M. Chan, *Chem. Soc. Rev.*, 2015, **44**, 1653–1679.
3 J. Owen, *Science*, 2015, **347**, 615–616.
4 M. D. Peterson, L. C. Cass, R. D. Harris, K. Edme, K. Sung and E. A. Weiss, *Annu. Rev. Phys. Chem.*, 2014, **65**, 317–339.
5 G. H. Carey, A. L. Abdelhady, Z. Ning, S. M. Thon, O. M. Bakr and E. H. Sargent, *Chem. Rev.*, 2015, **115**, 12732–12763.
6 D. V. Talapin, J.-S. Lee, M. V. Kovalenko and E. V. Shevchenko, *Chem. Rev.*, 2010, **110**, 389–458.
7 G. Chen, I. Roy, C. Yang and P. N. Prasad, *Chem. Rev.*, 2016, **116**, 2826–2885.
8 W. Wang, A. Kapur, X. Ji, B. Zeng, D. Mishra and H. Mattoussi, *Bioconjugate Chem.*, 2016, **27**, 2024–2036.
9 M. C. Weidman, M. E. Beck, R. S. Hoffman, F. Prins and W. A. Tisdale, *ACS Nano*, 2014, **8**, 6363–6371.
10 P. D. Jadzinsky, G. Calero, C. J. Ackerson, D. A. Bushnell and R. D. Kornberg, *Science*, 2007, **318**, 430–433.
11 M. Azubel and R. D. Kornberg, *Nano Lett.*, 2016, **16**, 3348–3351.
12 K. E. Knowles, M. T. Frederick, D. B. Tice, A. J. Morris-Cohen and E. A. Weiss, *J. Phys. Chem. Lett.*, 2012, **3**, 18–26.
13 E. A. Weiss, *Acc. Chem. Res.*, 2013, **46**, 2607–2615.

14 M. A. Boles, D. Ling, T. Hyeon and D. V. Talapin, *Nat. Mater.*, 2016, **15**, 141–153.
15 M. M. Krause and P. Kambhampati, *Phys. Chem. Chem. Phys.*, 2015, **17**, 18882–18894.
16 A. J. Morris-Cohen, M. Malicki, M. D. Peterson, J. W. J. Slavin and E. A. Weiss, *Chem. Mater.*, 2013, **25**, 1155–1165.
17 P. Reiss, M. Protière and L. Li, *Small*, 2009, **5**, 154–168.
18 C. Bullen and P. Mulvaney, *Langmuir*, 2006, **22**, 3007–3013.
19 A. M. Smith, H. W. Duan, M. N. Rhyner, G. Ruan and S. M. Nie, *Phys. Chem. Chem. Phys.*, 2006, **8**, 3895–3903.
20 Y. Shen, M. Y. Gee, R. Tan, P. J. Pellechia and A. B. Greytak, *Chem. Mater.*, 2013, **25**, 2838–2848.
21 R. Tan, Y. Shen, S. K. Roberts, M. Y. Gee, D. A. Blom and A. B. Greytak, *Chem. Mater.*, 2015, **27**, 7468–7480.
22 N. C. Anderson, M. P. Hendricks, J. J. Choi and J. S. Owen, *J. Am. Chem. Soc.*, 2013, **135**, 18536–18548.
23 A. Hassinen, I. Moreels, K. De Nolf, P. F. Smet, J. C. Martins and Z. Hens, *J. Am. Chem. Soc.*, 2012, **134**, 20705–20712.
24 B. Shakeri and R. W. Meulenberg, *Langmuir*, 2015, **31**, 13433–13440.
25 B. Kowalczyk, I. Lagzi and B. A. Grzybowski, *Curr. Opin. Colloid Interface Sci.*, 2011, **16**, 135–148.
26 J. S. Beveridge, M. R. Buck, J. F. Bondi, R. Misra, P. Schiffer, R. E. Schaak and M. E. Williams, *Angew. Chem., Int. Ed.*, 2011, **50**, 9875–9879.
27 G. Kalyuzhny and R. W. Murray, *J. Phys. Chem. B*, 2005, **109**, 7012–7021.
28 A. M. Munro, I. Jen-La Plante, M. S. Ng and D. S. Ginger, *J. Phys. Chem. C*, 2007, **111**, 6220–6227.
29 Y. Shen, R. Tan, M. Y. Gee and A. B. Greytak, *ACS Nano*, 2015, **9**, 3345–3359.
30 A. B. Nepomnyashchii, R. D. Harris and E. A. Weiss, *Anal. Chem.*, 2016, **88**, 3310–3316.
31 G. M. Carroll, C. K. Brozek, K. H. Hartstein, E. Y. Tsui and D. R. Gamelin, *J. Am. Chem. Soc.*, 2016, **138**, 4310–4313.
32 E. S. Williams, K. J. Major, A. Tobias, D. Woodall, V. Morales, C. Lippincott, P. J. Moyer and M. Jones, *J. Phys. Chem. C*, 2013, **117**, 4227–4237.
33 X. Li, V. M. Nichols, D. Zhou, C. Lim, G. S. H. Pau, C. J. Bardeen and M. L. Tang, *Nano Lett.*, 2014, **14**, 3382–3387.
34 A. Widmer-Cooper and P. L. Geissler, *ACS Nano*, 2016, **10**, 1877–1887.
35 R. R. Knauf, J. C. Lennox and J. L. Dempsey, *Chem. Mater.*, 2016, **28**, 4762–4770.
36 J. L. Stein, E. A. Mader and B. M. Cossairt, *J. Phys. Chem. Lett.*, 2016, **7**, 1315–1320.
37 T. Akdas, J. Walter, D. Segets, M. Distaso, B. Winter, B. Birajdar, E. Spiecker and W. Peukert, *Nanoscale*, 2015, **7**, 18105–18118.
38 T. Akdas, M. Distaso, S. Kuhri, B. Winter, B. Birajdar, E. Spiecker, D. M. Guldi and W. Peukert, *J. Colloid Interface Sci.*, 2015, **445**, 337–347.
39 A. R. Kortan, R. Hull, R. L. Opila, M. G. Bawendi, M. L. Steigerwald, P. J. Carroll and L. E. Brus, *J. Am. Chem. Soc.*, 1990, **112**, 1327–1332.
40 C. B. Murray, D. J. Norris and M. G. Bawendi, *J. Am. Chem. Soc.*, 1993, **115**, 8706–8715.
41 T. Vossmeyer, L. Katsikas, M. Giersig, I. G. Popovic, K. Diesner, A. Chemseddine, A. Eychmueller and H. Weller, *J. Phys. Chem.*, 1994, **98**, 7665–7673.
42 N. Gaponik, D. V. Talapin, A. L. Rogach, K. Hoppe, E. V. Shevchenko, A. Kornowski, A. Eychmüller and H. Weller, *J. Phys. Chem. B*, 2002, **106**, 7177–7185.
43 P. Yu, M. C. Beard, R. J. Ellingson, S. Ferrere, C. Curtis, J. Drexler, F. Luiszer and A. J. Nozik, *J. Phys. Chem. B*, 2005, **109**, 7084–7087.
44 A. Cros-Gagneux, F. Delpech, C. Nayral, A. Cornejo, Y. Coppel and B. Chaudret, *J. Am. Chem. Soc.*, 2010, **132**, 18147–18157.
45 H. Virieux, M. Le Troedec, A. Cros-Gagneux, W.-S. Ojo, F. Delpech, C. Nayral, H. Martinez and B. Chaudret, *J. Am. Chem. Soc.*, 2012, **134**, 19701–19708.
46 A. J. Morris-Cohen, M. D. Donakowski, K. E. Knowles and E. A. Weiss, *J. Phys. Chem. C*, 2010, **114**, 897–906.
47 A. J. Morris-Cohen, M. T. Frederick, G. D. Lilly, E. A. McArthur and E. A. Weiss, *J. Phys. Chem. Lett.*, 2010, **1**, 1078–1081.
48 X. Peng, T. E. Wilson, A. P. Alivisatos and P. G. Schultz, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 145–147.
49 T. X. Ding, J. H. Olshansky, S. R. Leone and A. P. Alivisatos, *J. Am. Chem. Soc.*, 2015, **137**, 2021–2029.
50 L. C. Cass, M. Malicki and E. A. Weiss, *Anal. Chem.*, 2013, **85**, 6974–6979.
51 A. M. Smith, A. M. Mohs and S. Nie, *Nat. Nanotechnol.*, 2009, **4**, 56–63.
52 W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem. Mater.*, 2003, **15**, 2854–2860.
53 W. W. Yu and X. Peng, *Angew. Chem., Int. Ed.*, 2002, **41**, 2368–2371.
54 Y. Yang, J. Li, L. Lin and X. Peng, *Nano Res.*, 2015, **8**, 3353–3364.
55 W. Nan, Y. Niu, H. Qin, F. Cui, Y. Yang, R. Lai, W. Lin and X. Peng, *J. Am. Chem. Soc.*, 2012, **134**, 19685–19693.
56 Z. Li, Y. Ji, R. Xie, S. Y. Grisham and X. Peng, *J. Am. Chem. Soc.*, 2011, **133**, 17248–17256.
57 C. Pu, J. Zhou, R. Lai, Y. Niu, W. Nan and X. Peng, *Nano Res.*, 2013, **6**, 652–670.
58 J. Liu, R. Liu, Y. Yin and G. Jiang, *Chem. Commun.*, 2009, 1514–1516.
59 M. Ganguly, A. Pal and T. Pal, *J. Phys. Chem. C*, 2012, **116**, 9265–9273.
60 S. A. Claridge, S. L. Goh, J. M. J. Fréchet, S. C. Williams, C. M. Micheel and A. P. Alivisatos, *Chem. Mater.*, 2005, **17**, 1628–1635.
61 R. A. Sperling, T. Pellegrino, J. K. Li, W. H. Chang and W. J. Parak, *Adv. Funct. Mater.*, 2006, **16**, 943–948.
62 M. Howarth, W. Liu, S. Puthenveetil, Y. Zheng, L. F. Marshall, M. M. Schmidt, K. D. Wittrup, M. G. Bawendi and A. Y. Ting, *Nat. Methods*, 2008, **5**, 397–399.
63 W. Liu, A. B. Greytak, J. Lee, C. R. Wong, J. Park, L. F. Marshall, W. Jiang, P. N. Curtin, A. Y. Ting, D. G. Nocera, D. Fukumura, R. K. Jain and M. G. Bawendi, *J. Am. Chem. Soc.*, 2010, **132**, 472–483.
64 I. Arnaud, J.-P. Abid, C. Roussel and H. H. Girault, *Chem. Commun.*, 2005, 787–788.
65 M. Hanauer, S. Pierrat, I. Zins, A. Lotz and C. Sönnichsen, *Nano Lett.*, 2007, **7**, 2881–2885.
66 S. Ho, K. Critchley, G. D. Lilly, B. Shim and N. A. Kotov, *J. Mater. Chem.*, 2009, **19**, 1390–1394.
67 S. Knoppe, J. Boudon, I. Dolamic, A. Dass and T. Bürgi, *Anal. Chem.*, 2011, **83**, 5056–5061.
68 E. Lhuillier, P. Hease, S. Ithurria and B. Dubertret, *Chem. Mater.*, 2014, **26**, 4514–4520.
69 J. D. Bass, X. Ai, A. Bagabas, P. M. Rice, T. Topuria, J. C. Scott, F. H. Alharbi, H.-C. Kim, Q. Song and R. D. Miller, *Angew. Chem., Int. Ed.*, 2011, **50**, 6538–6542.
70 D. Kim, H. K. Park, H. Choi, J. Noh, K. Kim and S. Jeong, *Nanoscale*, 2014, **6**, 14467–14472.
71 J. W. Williams, K. E. Van Holde, R. L. Baldwin and H. Fujita, *Chem. Rev.*, 1958, **58**, 715–744.
72 M. S. Arnold, A. A. Green, J. F. Hulvat, S. I. Stupp and M. C. Hersam, *Nat. Nanotechnol.*, 2006, **1**, 60–65.
73 J. B. Miller, J. M. Harris and E. K. Hobbie, *Langmuir*, 2014, **30**, 7936–7946.
74 N. Stürzl, F. Hennrich, S. Lebedkin and M. M. Kappes, *J. Phys. Chem. C*, 2009, **113**, 14628–14632.
75 M. Calabretta, J. A. Jamison, J. C. Falkner, Y. Liu, B. D. Yuhas, K. S. Matthews and V. L. Colvin, *Nano Lett.*, 2005, **5**, 963–967.
76 J. A. Jamison, K. M. Krueger, C. T. Yavuz, J. T. Mayo, D. LeCrone, J. J. Redden and V. L. Colvin, *ACS Nano*, 2008, **2**, 311–319.
77 G. Chen, Y. Wang, L. H. Tan, M. Yang, L. S. Tan, Y. Chen and H. Chen, *J. Am. Chem. Soc.*, 2009, **131**, 4218–4219.
78 S. Li, Z. Chang, J. Liu, L. Bai, L. Luo and X. Sun, *Nano Res.*, 2011, **4**, 723–728.
79 J. P. Novak, C. Nickerson, S. Franzen and D. L. Feldheim, *Anal. Chem.*, 2001, **73**, 5758–5761.
80 F. Bonaccorso, M. Zerbetto, A. C. Ferrari and V. Amendola, *J. Phys. Chem. C*, 2013, **117**, 13217–13229.
81 X. Ma, Y. Kuang, L. Bai, Z. Chang, F. Wang, X. Sun and D. G. Evans, *ACS Nano*, 2011, **5**, 3242–3249.
82 E. E. Lees, M. J. Gunzburg, T.-L. Nguyen, G. J. Howlett, J. Rothacker, E. C. Nice, A. H. A. Clayton and P. Mulvaney, *Nano Lett.*, 2008, **8**, 2883–2890.
83 M. L. Mastronardi, F. Hennrich, E. J. Henderson, F. Maier-Flaig, C. Blum, J. Reichenbach, U. Lemmer, C. Kübel, D. Wang, M. M. Kappes and G. A. Ozin, *J. Am. Chem. Soc.*, 2011, **133**, 11928–11931.
84 Ž. Krpetić, A. M. Davidson, M. Volk, R. Lévy, M. Brust and D. L. Cooper, *ACS Nano*, 2013, **7**, 8881–8890.
85 V. Sharma, K. Park and M. Srinivasarao, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 4981–4985.
86 B. Xiong, J. Cheng, Y. Qiao, R. Zhou, Y. He and E. S. Yeung, *J. Chromatogr. A*, 2011, **1218**, 3823–3829.
87 R. Liu, J.-H. Zhou, Z.-K. Zhou, X. Jiang, J. Liu, G. Liu and X.-H. Wang, *Nanoscale*, 2014, **6**, 13145–13153.

88 O. Akbulut, C. R. Mace, R. V. Martinez, A. A. Kumar, Z. Nie, M. R. Patton and G. M. Whitesides, *Nano Lett.*, 2012, **12**, 4060–4064.
89 P. Li, J. Huang, L. Luo, Y. Kuang and X. Sun, *Anal. Chem.*, 2016, **88**, 8495–8501.
90 O. Carion, B. Mahler, T. Pons and B. Dubertret, *Nat. Protoc.*, 2007, **2**, 2383–2390.
91 A. M. Prantner, J. Chen, C. B. Murray and N. Scholler, *Chem. Mater.*, 2012, **24**, 4008–4010.
92 K. E. Sapsford, K. M. Tyner, B. J. Dair, J. R. Deschamps and I. L. Medintz, *Anal. Chem.*, 2011, **83**, 4453–4488.
93 L. Bai, X. Ma, J. Liu, X. Sun, D. Zhao and D. G. Evans, *J. Am. Chem. Soc.*, 2010, **132**, 2333–2337.
94 A. M. Alkilany, S. R. Abulateefeh, K. K. Mills, A. I. Bani Yaseen, M. A. Hamaly, H. S. Alkhatib, K. M. Aiedeh and J. W. Stone, *Langmuir*, 2014, **30**, 13799–13808.
95 C. Dong, H. Qian, N. Fang and J. Ren, *J. Phys. Chem. B*, 2006, **110**, 11069–11075.
96 X. Zhang, A. Shamirian, A. M. Jawaid, C. M. Tyrakowski, L. E. Page, A. Das, O. Chen, A. Isovic, A. Hassan and P. T. Snee, *Small*, 2015, **11**, 6091–6096.
97 Y. Xing, Q. Chaudry, C. Shen, K. Y. Kong, H. E. Zhau, L. W. Chung, J. A. Petros, R. M. O'Regan, M. V. Yezhelyev, J. W. Simons, M. D. Wang and S. Nie, *Nat. Protoc.*, 2007, **2**, 1152–1165.
98 W. Liu, H. S. Choi, J. P. Zimmer, E. Tanaka, J. V. Frangioni and M. Bawendi, *J. Am. Chem. Soc.*, 2007, **129**, 14530–14531.
99 A. Viswanath, Y. Shen, A. N. Green, R. Tan, A. B. Greytak and B. C. Benicewicz, *Macromolecules*, 2014, **47**, 8137–8144.
100 P. T. Snee, R. C. Somers, G. Nair, J. P. Zimmer, M. G. Bawendi and D. G. Nocera, *J. Am. Chem. Soc.*, 2006, **128**, 13320–13321.
101 M. M. Mekawy, A. Yamaguchi, S. A. El-Safty, T. Itoh and N. Teramae, *J. Colloid Interface Sci.*, 2011, **355**, 348–358.
102 S. A. El-Safty, M. Mekawy, A. Yamaguchi, A. Shahat, K. Ogawa and N. Teramae, *Chem. Commun.*, 2010, **46**, 3917–3919.
103 T. R. Gaborski, J. L. Snyder, C. C. Striemer, D. Z. Fang, M. Hoffman, P. M. Fauchet and J. L. McGrath, *ACS Nano*, 2010, **4**, 6973–6981.
104 E. Krieg, H. Weissman, E. Shirman, E. Shimoni and B. Rybtchinski, *Nat. Nanotechnol.*, 2011, **6**, 141–146.
105 N. Alele, R. Streubel, L. Gamrad, S. Barcikowski and M. Ulbricht, *Sep. Purif. Technol.*, 2016, **157**, 120–130.
106 N. Alele and M. Ulbricht, *Sep. Purif. Technol.*, 2016, **158**, 171–182.
107 S. F. Sweeney, G. H. Woehrle and J. E. Hutchison, *J. Am. Chem. Soc.*, 2006, **128**, 3190–3197.
108 E. R. Goldman, G. P. Anderson, P. T. Tran, H. Mattoussi, P. T. Charles and J. M. Mauro, *Anal. Chem.*, 2002, **74**, 841–847.
109 R. Lévy, Z. Wang, L. Duchesne, R. C. Doty, A. I. Cooper, M. Brust and D. G. Fernig, *ChemBioChem*, 2006, **7**, 592–594.
110 K. Tiede, A. B. A. Boxall, D. Tiede, S. P. Tear, H. David and J. Lewis, *J. Anal. At. Spectrom.*, 2009, **24**, 964–972.
111 S. A. Pergantis, T. L. Jones-Lepp and E. M. Heithmar, *Anal. Chem.*, 2012, **84**, 6454–6462.
112 N. Yan, Z. Zhu, L. Jin, W. Guo, Y. Gan and S. Hu, *Anal. Chem.*, 2015, **87**, 6079–6087.
113 A. M. Al-Somali, K. M. Krueger, J. C. Falkner and V. L. Colvin, *Anal. Chem.*, 2004, **76**, 5903–5910.
114 I. Dolamic, S. Knoppe, A. Dass and T. Bürgi, *Nat. Commun.*, 2012, **3**, 798.
115 S. Knoppe, M. Vanbel, S. van Cleuvenbergen, L. Vanpraet, T. Bürgi and T. Verbiest, *J. Phys. Chem. C*, 2015, **119**, 6221–6226.
116 Y. Shen, A. Roberge, R. Tan, M. Y. Gee, D. C. Gary, Y. Huang, D. A. Blom, B. C. Benicewicz, B. M. Cossairt and A. B. Greytak, *Chem. Sci.*, 2016, **7**, 5671–5679.
117 J. P. Wilcoxon, J. E. Martin and P. Provencio, *Langmuir*, 2000, **16**, 9912–9920.
118 M. C. S. Pierre and A. J. Haes, *Anal. Chem.*, 2012, **84**, 7906–7911.
119 A. K. Brewer and A. M. Striegel, *Anal. Chem.*, 2011, **83**, 3068–3075.
120 A. López-Serrano, R. M. Olivas, J. S. Landaluze and C. Cámara, *Anal. Methods*, 2013, **6**, 38–56.
121 T. Rheinländer, R. Kötitz, W. Weitschies and W. Semmler, *Colloid Polym. Sci.*, 2000, **278**, 259–263.
122 K. M. Krueger, A. M. Al-Somali, J. C. Falkner and V. L. Colvin, *Anal. Chem.*, 2005, **77**, 3511–3515.
123 J. P. Wilcoxon and P. P. Provencio, *J. Phys. Chem. B*, 2005, **109**, 13461–13471.
124 S. A. Owens, M. C. Carpenter, J. W. H. Sonne, C. A. Miller, J. R. Renehan, C. A. Odonkor, E. M. Henry and D. T. Miles, *J. Phys. Chem. C*, 2011, **115**, 18952–18957.
125 L. Alamo-Nole, S. Bailon-Ruiz, O. Perales-Perez and F. R. Roman, *Anal. Methods*, 2012, **4**, 3127–3132.
126 T. Arita, T. Yoshimura and T. Adschiri, *Nanoscale*, 2010, **2**, 1467–1473.
127 S.-Y. Ding, M. Jones, M. P. Tucker, J. M. Nedeljkovic, J. Wall, M. N. Simon, G. Rumbles and M. E. Himmel, *Nano Lett.*, 2003, **3**, 1581–1585.
128 L. Pitkänen and A. M. Striegel, *Anal. Bioanal. Chem.*, 2016, **408**, 4003–4010.
129 M. S. Reisch, *Chem. Eng. News*, 2016, **94**, 35–36.
130 I. Kanelidis, A. Vaneski, D. Lenkeit, S. Pelz, V. Elsner, R. M. Stewart, J. Rodríguez-Fernández, A. A. Lutich, A. S. Susha, R. Theissmann, S. Adamczyk, A. L. Rogach and E. Holder, *J. Mater. Chem.*, 2011, **21**, 2656–2662.
131 C. Tang, J. Tan, J. Jin, S. Xi, H. Li, Q. Xie and X. Peng, *Rapid Commun. Mass Spectrom.*, 2015, **29**, 1863–1873.
132 M. Wang, G. R. Bardajee, S. Kumar, M. Nitz, G. D. Scholes and M. A. Winnik, *J. Chromatogr. A*, 2009, **1216**, 5011–5019.
133 K. Davis, B. Qi, M. Witmer, C. L. Kitchens, B. A. Powell and O. T. Mefford, *Langmuir*, 2014, **30**, 10918–10925.
134 L. Ye, T. Pearson, C. Dolbashian, P. Pstrak, A. R. Mohtasebzadeh, B. Fellows, O. T. Mefford and T. M. Crawford, *Adv. Funct. Mater.*, 2016, **26**, 3983–3989.
135 J.-K. Wu, Z.-Q. Tian, Z.-L. Zhang, A.-A. Liu, B. Tang, L.-J. Zhang, Z.-L. Chen and D.-W. Pang, *Talanta*, 2016, **159**, 64–73.
136 X. Zhao, Y. Shen, E. A. Adogla, A. Viswanath, R. Tan, B. C. Benicewicz, A. B. Greytak, Y. Lin and Q. Wang, *J. Mater. Chem. B*, 2016, **4**, 2421–2427.
137 C. M. Johnson, K. M. Pate, Y. Shen, A. Viswanath, R. Tan, B. C. Benicewicz, M. A. Moss and A. B. Greytak, *J. Colloid Interface Sci.*, 2015, **458**, 310–314.
138 M. Wang, T. E. Dykstra, M. R. Salvador, X. Lou, G. D. Scholes and M. A. Winnik, *Angew. Chem., Int. Ed.*, 2006, **45**, 2221–2224.
139 W. Biesta, B. van Lagen, V. S. Gevaert, A. T. M. Marcelis, J. M. J. Paulusse, M. W. F. Nielen and H. Zuilhof, *Chem. Mater.*, 2012, **24**, 4311–4318.
140 J. P. Zimmer, S.-W. Kim, S. Ohnishi, E. Tanaka, J. V. Frangioni and M. G. Bawendi, *J. Am. Chem. Soc.*, 2006, **128**, 2526–2527.
141 H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi and J. V. Frangioni, *Nat. Biotechnol.*, 2007, **25**, 1165–1170.
142 W. Liu, M. Howarth, A. B. Greytak, Y. Zheng, D. G. Nocera, A. Y. Ting and M. G. Bawendi, *J. Am. Chem. Soc.*, 2008, **130**, 1274–1284.
143 E. J. McLaurin, A. B. Greytak, M. G. Bawendi and D. G. Nocera, *J. Am. Chem. Soc.*, 2009, **131**, 12994–13001.
144 L. Xie, Y. Shen, D. Franke, V. Sebastián, M. G. Bawendi and K. F. Jensen, *J. Am. Chem. Soc.*, 2016, **138**, 13469–13472.
145 D. Parobek, B. J. Roman, Y. Dong, H. Jin, E. Lee, M. Sheldon and D. H. Son, *Nano Lett.*, 2016, DOI: 10.1021/acs.nanolett.6b02772.
146 S. J. Kern, K. Sahu and M. A. Berg, *Nano Lett.*, 2011, **11**, 3493–3498.
147 M. M. Krause, L. Jethi, T. G. Mack and P. Kambhampati, *J. Phys. Chem. Lett.*, 2015, **6**, 4292–4296.
148 M. A. Petruska, A. V. Malko, P. M. Voyles and V. I. Klimov, *Adv. Mater.*, 2003, **15**, 610–613.
149 C. A. Leatherdale and M. G. Bawendi, *Phys. Rev. B*, 2001, **63**, 165315.
150 B. T. Diroll, T. Dadosh, A. Koschitzky, Y. E. Goldman and C. B. Murray, *J. Phys. Chem. C*, 2013, **117**, 23928–23937.
151 J. S. Owen, J. Park, P.-E. Trudeau and A. P. Alivisatos, *J. Am. Chem. Soc.*, 2008, **130**, 12279–12281.
152 J. De Roo, Y. Justo, K. De Keukeleere, F. Van den Broeck, J. C. Martins, I. Van Driessche and Z. Hens, *Angew. Chem., Int. Ed.*, 2015, **54**, 6488–6491.
153 F. Wang, R. Tang and W. E. Buhro, *Nano Lett.*, 2008, **8**, 3521–3524.
154 J. De Roo, K. D. Keukeleere, Z. Hens and I. V. Driessche, *Dalton Trans.*, 2016, **45**, 13277–13283.
155 M. Cirillo, F. Strubbe, K. Neyts and Z. Hens, *ACS Nano*, 2011, **5**, 1345–1352.
156 F. Knittel, E. Gravel, E. Cassette, T. Pons, F. Pillon, B. Dubertret and E. Doris, *Nano Lett.*, 2013, **13**, 5075–5078.
157 T. Uematsu, E. Shimomura, T. Torimoto and S. Kuwabata, *J. Phys. Chem. C*, 2016, **120**, 16012–16023.

158 R. Dierick, F. Van den Broeck, K. De Nolf, Q. Zhao, A. Vantomme, J. C. Martins and Z. Hens, *Chem. Mater.*, 2014, **26**, 5950–5957.
159 M. J. Turo and J. E. Macdonald, *ACS Nano*, 2014, **8**, 10205–10213.
160 M. J. Turo, X. Shen, N. K. Brandon, S. Castillo, A. M. Fall, S. T. Pantelides and J. E. Macdonald, *Chem. Commun.*, 2016, **52**, 12214–12217.
161 B. Fritzinger, R. K. Capek, K. Lambert, J. C. Martins and Z. Hens, *J. Am. Chem. Soc.*, 2010, **132**, 10195–10201.
162 D. C. Gary and B. M. Cossairt, *Chem. Mater.*, 2013, **25**, 2463–2469.
163 S. F. Wuister, C. de Mello Donegá and A. Meijerink, *J. Phys. Chem. B*, 2004, **108**, 17393–17397.
164 A. M. Munro and D. S. Ginger, *Nano Lett.*, 2008, **8**, 2585–2590.
165 M. A. Hines and P. Guyot-Sionnest, *J. Phys. Chem.*, 1996, **100**, 468–471.
166 L. Hartmann, A. Kumar, M. Welker, A. Fiore, C. Julien-Rabant, M. Gromova, M. Bardet, P. Reiss, P. N. W. Baxter, F. Chandezon and R. B. Pansu, *ACS Nano*, 2012, **6**, 9033–9041.
167 Y. Gao and X. Peng, *J. Am. Chem. Soc.*, 2015, **137**, 4230–4235.
168 C. Pu and X. Peng, *J. Am. Chem. Soc.*, 2016, **138**, 8134–8142.
169 L. Deng, X. Wang, Y. Kuang, C. Wang, L. Luo, F. Wang and X. Sun, *Nano Res.*, 2015, **8**, 2810–2821.
170 D. C. Gary, M. W. Terban, S. J. L. Billinge and B. M. Cossairt, *Chem. Mater.*, 2015, **27**, 1432–1441.
171 D. Zherebetskyy, M. Scheele, Y. Zhang, N. Bronstein, C. Thompson, D. Britt, M. Salmeron, P. Alivisatos and L.-W. Wang, *Science*, 2014, **344**, 1380–1384.
172 M. A. White, M. J. Thompson, G. J. Miller and J. Vela, *Chem. Commun.*, 2016, **52**, 3497–3499.
173 C. J. Murphy and J. M. Buriak, *Chem. Mater.*, 2015, **27**, 4911–4913.