Preparation of chiral quantum dots

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Chiral quantum dots (QDs) are expected to have a range of potential applications in photocatalysis, as specific antibacterial and cytotoxic drug-delivery agents, in assays, as sensors in asymmetric synthesis and enantioseparation, and as fluorescent chiral nanoprobes in biomedical and analytical technologies. In this protocol, we present procedures for the synthesis of chiral optically active QD nanostructures and their quality control using spectroscopic studies and transmission electron microscopy imaging. We closely examine various synthetic routes for the preparation of chiral CdS, CdSe, CdTe and doped ZnS QDs, as well as of chiral CdS nanotetrapods. Most of these nanomaterials can be produced by a very fast (70 s) microwave-induced heating of the corresponding precursors in the presence of D- or L-chiral stabilizing coating ligands (stabilizers), which are crucial to generating optically active chiral QDs. Alternatively, chiral QDs can also be produced via the conventional hot injection technique, followed by a phase transfer in the presence of an appropriate chiral stabilizer. We demonstrate that the properties, structure and behavior of chiral QD nanostructures, as determined by various spectroscopic techniques, strongly depend on chiral stabilizers and that the chiral effects induced by them can be controlled via synthetic procedures.

INTRODUCTION

Symmetry and chirality are properties commonly found throughout the natural world. Chirality is one of the most important factors in molecular recognition, with chiral compounds having a major role in chemistry, biology and medicine. Chirality has also been envisaged to have an important role in nanotechnology[1–6], and an understanding of the fundamental concepts relevant to chirality in nanosystems is important for the further advancement of nanoscience in general and for nanobiotechnology in particular.

Over the past years, the research on chiral metal nanoparticles has received a great deal of attention owing to the range of potential applications offered by these materials as chiral sensors, catalysts and as metamaterials in advanced optical devices[1–3,7–11]. The use of stereospecific chiral stabilizing molecules has also opened up another avenue of interest in the area of QD research. Optically active chiral QDs (penicillamine-stabilized CdS) were first prepared by our group by using microwave-induced heating with the racemic (*Rac*), D- and L-enantiomeric forms of penicillamine used as stabilizers[12]. It was found that these QDs demonstrated very broad luminescence bands (between 370 and 710 nm), which are attributed to defects or electron-trap states on the surface of the nanocrystals. Importantly, a clear relationship between luminescence that originated by defects and circular dichroism (CD) activity was observed for CdS chiral QDs. Our density functional calculations of the electronic states have demonstrated that CD at longer wavelengths is associated with near-surface cadmium atoms that are enantiomerically distorted by chiral penicillamine ligands, which translate their enantiomeric structure to the surface layers and associated electronic states, whereas the QD core is found to remain undistorted and achiral[13].

After that work, we later reported the preparation of chiral CdSe (ref. 14), CdTe (refs. 15,16) and chiral CdS nanotetrapods[17]. All of these chiral nanostructures showed characteristic CD responses within the band-edge region of their UV-visible spectrum, as well as very broad distribution of photoluminescence (PL), which originates from emissive defect states. The concept of chiral surface defects of QDs (distorted QD shell) was also confirmed by other groups with CdTe nanocrystals bearing various chiral ligands[18–20]. Interestingly, it was shown that the chirality of the QD surface was maintained even after ligand exchange with an achiral thiol and subsequent transfer of the CdTe QDs into a different (organic) phase. In this case, chiral QDs have shown a unique 'chiral memory' effect[19].

However, chirality in chiral QDs can be caused not necessarily only by chiral surface defects but also by other factors. For example, the effects of cysteine enantiomers on optical isomerism, growth rate and structure of chiral CdTe–based QDs was reported by Kotov and colleagues[20]. This paper postulated that the atomic origin of chiral sites in nanoparticles is geometrically similar to that in organic compounds. By using theoretical calculations and experimental data, the researchers showed that atoms in chiral cysteine–stabilized CdTe nanocrystals are arranged as tetrahedrons, and that chirality occurs when all tetrahedral apexes have chemical differences and substitution[20].

More recently, chiral ligand–induced CD in CdSe QDs was reported by Balaz and colleagues[21,22]. The researchers have found that a simple phase transfer of trioctylphosphine oxide or oleic acid–capped CdSe QDs from toluene into aqueous phase using chiral thiol capping ligands such as L- and D-cysteines can induce chiroptical properties in originally achiral cadmium selenide QDs. In addition, it was shown that L- or D-cysteine-stabilized QDs in aqueous phase demonstrated size-dependent electronic CD and chiral ligand–induced circularly polarized luminescence in QDs. In this case, the authors have explained the origin of the induced CD in QDs by the hybridization of the chiral ligand's highest occupied molecular orbitals (HOMOs) with CdSe molecular orbitals[21].

The literature data above clearly demonstrate that chiral stabilizing ligands and induced chirality effects have a crucial role in the properties and behavior of chiral QDs, enabling a range of potential applications for these nanomaterials in chemistry, nanotechnology and biology.

One important application of chiral QDs is luminescence sensing and chiral recognition of enantiomers[18,23,24]. A fundamental

fact is that QDs can change their fluorescence properties (e.g., emission intensity, peak position and luminescence lifetime) depending on the environment. This change occurs owing to the energy transfer and other processes taking place between QDs and the surrounding molecular species. Therefore, any stereospecific interaction of chiral QDs with selected enantiomeric molecules (e.g. drugs and biomolecules) should result in a luminescence response. For example, it was demonstrated that the fluorescence intensity of L-cysteine-stabilized chiral CdSe/ZnS core/shell QDs decayed in the presence of D-carnitine but were not affected by L-carnitine, whereas the fluorescence of D-cysteine-modified QDs was only affected by L-carnitine[24]. This chiral recognition behavior was interpreted as a preferential interaction between a D-carnitine enantiomer with a chiral L-cysteine-capped nanoparticle surface and vice versa. In addition, our studies have also shown that chiral CdSe QDs demonstrate enantioselective chiral recognition of selected amino acids and DNA[23]. In these experiments, the addition of calf thymus DNA resulted in significant decreases in the emission intensities of D-penicillamine–stabilized CdSe QDs, whereas the emission of corresponding L-penicillamine–stabilized QDs was not affected. More recently, it was reported that chiral CdSe/ZnS QDs capped with *N*-acetyl-L-cysteine methyl ester can be used for sensing of various chiral organic drug molecules such as the aryl propionic acids ketoprofen, naproxen, flurbiprofen and ibuprofen[25]. It was found that all of the drug molecules quenched the QD emission in a concentration-dependent manner. The spectral differences in the behavior of *R*- and *S*-enantiomers of these aryl propionic acid drugs enabled the quantitative determination of both chiral forms in mixtures and pharmaceutical samples. Thus, as mentioned earlier, chiral QDs have great potential to serve as assays, nanoprobes and optical sensors in asymmetric synthesis, enantioseparation, biomedical analytical technologies and medical diagnostics. In addition, chiral QDs with an appropriate functionality could potentially serve as specific antibacterial and cytotoxic drug-delivery agents, as well as new enantioselective catalysts. For example, it was recently reported that ZnS QDs with an induced chirality were used as catalysts for asymmetric aldol condensation reactions[26]. There was also a recent publication on the investigation of the coupling of optical activity in the binary assemblies containing gold nanorods and chiral CdTe QDs[27]. It was demonstrated that the optical coupling between chiral QDs and Au nanorods results in the generation of a plasmonic CD response and the simultaneous enhancement of the CD intensity from the QDs. It was shown that the optical activity of these assemblies can be controlled by changing either the aspect ratio of gold nanorods or the size of QDs[27]. These new chiral QD-metal nanoparticle assemblies are expected to find a range of potential applications, including their use as metamaterials, circular light polarizers and sensors.

The main aim of the protocol herein is to provide detailed procedures for the synthesis and characterization of chiral QD-based nanostructures, which may be used by scientists from a variety of disciplines. In this paper, we will present and closely examine nonhazardous routes for the preparation of chiral II–VI (comprising elements of groups II and VI of the periodic table) semiconducting nanocrystals. Initially, we outline procedures that are used for the synthesis of chiral optically active CdS, CdSe and CdTe QDs[12,13,14,23]. These chiral nanoparticles have been prepared using various enantiomers of penicillamine and cysteine as stabilizers.

We then describe the synthesis of CdS and Mn-doped CdS nanotetrapods. Nonchiral tetrapodal QD nanostructures have been previously reported and prepared through organic, high-temperature injection techniques[28–32]. By contrast, our approach enables us to prepare chiral optically active tetrapodal quantum nanostructures via an aqueous method at much lower temperature than previously reported.

Finally, we present a new procedure using an alternative synthetic route for the preparation of chiral CdS and manganese-doped ZnS (Mn:ZnS)-based QDs. This involves a traditional hot injection technique in an appropriate organic solvent, followed by an aqueous phase transfer using chiral cysteine or penicillamine ligands and heating. It is a very robust and cost-effective synthetic approach that enables users to achieve a relatively high yield of chiral QD products.

Limitations regarding synthesis and characterization of the chiral optically active QDs

Although the II–VI QDs prepared by microwave synthesis can be easily characterized by spectroscopic techniques such as UV-visible, PL or CD, frequently they are not easily identified using transmission electron microscopy (TEM). These nanoparticles are quite small, and it is very difficult to see the lattice fringes in high-resolution TEM. This is probably due to their low degree of crystallinity and the presence of a large number of defects. The emission and optical activity of these nanoparticles (ANTICIPATED RESULTS) have been demonstrated to be attributable to defect states present within the QD. As a result, it may be expected that the precise extent of optical and photophysical properties may not be exactly replicated for essentially identical synthetic procedures, as they are the result of imperfections in the nanoparticle and not the overall nanoparticle, which may have been reproduced. Potentially, the defects can be removed by heating these QDs under reflux, in a Cd^{2+}/stabilizer medium, resulting in intrinsically emitting dots. However, this treatment will also remove the optical activity and CD responses of the QDs.

Other limitations of the synthesis of chiral cadmium–based QDs include the following:

- A number of the reagents (e.g., Cd salts) are highly toxic, and they must be handled very carefully.
- The yields in all aqueous synthetic approaches (e.g., microwave and under reflux heating synthesis of CdS) are very low.
- Some organic reagents are somewhat oxygen sensitive once solubilized, especially thioacetamide, and as a result the stock solutions must be used within at most 1 d of being produced.
- During the QD cleaning process, particle aggregation can occur as a result of either chemical bonding between the ligand spheres of nanoparticles or the effect of high surface energy leading to the joining of particles to bulk-sized materials.
- Hot injection synthesis of CdS and Mn-doped ZnS particles requires high temperatures (up to 315 °C), and it involves large amounts of sulfur-generating poisonous gases in the process, thus requiring careful consideration and handling.

Experimental design

The aqueous optically active chiral CdS nanocrystals reported here were prepared using a method initially reported by Ni and

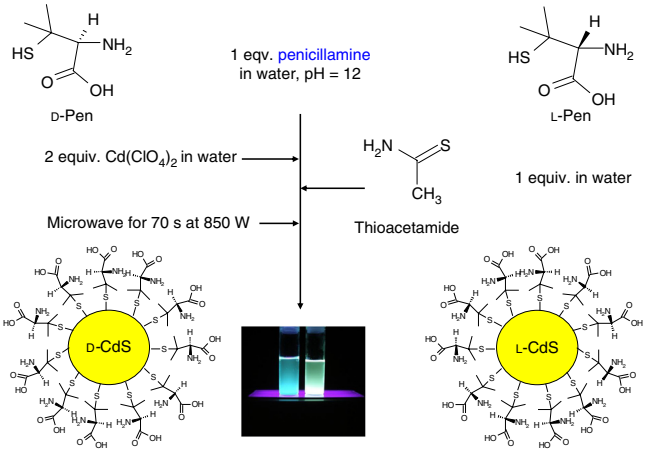

Figure 1 | Schematic presentation of the synthesis of chiral CdS QDs.

colleagues[33], which has been modified by us using chiral penicillamine stabilizers. The general scheme of the synthesis is presented in **Figure 1**. In our procedure, aqueous stock solutions of cadmium perchlorate, thioacetamide (CH_3CSNH_2), and basic aqueous D-, L- or racemic mixture (*Rac*) penicillamine-stabilizer solutions are prepared. The chiral stabilizer, cadmium perchlorate and thioacetamide stock solutions are then added in sequence and under magnetic stirring to the chiral stabilizer solution in water. The pH of the resulting solution is adjusted to 12 using sodium hydroxide $(NaOH)_{(aq)}$. This precursor mixture is then placed in the CEM Star System 6 microwave and heated for 70 s at 850 W. The vessel containing the freshly prepared particles is removed from the microwave, and its contents are transferred to a round-bottomed flask (RBF). The flask is then wrapped in aluminum foil to exclude light, and it is placed in a cool, dark place and allowed to mature for at least 24 h. This method of reaction initiation ensures that the thioacetamide (S^{2-} source) decomposes in such a way as to release sulfide ions into solution as quickly and uniformly as possible. This is done to allow for the instantaneous formation of numerous nucleation centers. These nucleation centers continue to uniformly grow until no S^{2-} ions remain, and it results in a monodisperse colloid. A rotary evaporator was then used to reduce the volume of the colloid, and the particles were precipitated out using propan-2-ol. The particles are then re-dispersed in water, and this process is normally repeated several more times until the particles are well washed and purified. After purification, the QDs can be re-dispersed in water, and they are ready for use and further studies.

Defect-emitting chiral CdSe dots can be prepared analogously to the CdS QDs. However, in this case, CH_3CSNH_2 (thioacetamide) is substituted for Na_2SeSO_3 as the Se^{2-} source[34,35]. Water-soluble Na_2SeSO_3 can be prepared using the method reported by Bhuse *et al.*[36], and it should be used immediately after its preparation. In our procedure, a basic aqueous solution of stabilizer (D-, L- or *Rac*) with the pH adjusted to 12 is prepared along with stock solutions of $Cd(ClO_4)_2{\cdot}xH_2O$ and Na_2SeSO_3. These solutions are then added in sequence to 45 ml of degassed Millipore water. The pH is re-adjusted to 12. The subsequent homogeneous solution is then placed into a CEM Star System 6 and irradiated for 70 s at 850 W. The resulting clear, yellow solution is then stored in the dark to mature for at least 24 h. The washing and purification of CdSe QDs can be performed similarly to CdS QDs above.

Microwave irradiation has previously been used to produce highly luminescent CdTe nanocrystals[37,38]. Here we prepare chiral CdTe QDs using a modified version of the technique reported by Bao *et al.*[39]. We have modified the synthesis of CdTe QDs to resemble the earlier CdS and CdSe preparation as closely as possible. Na_2TeO_3 takes the place of CH_3CSNH_2 and Na_2SeSO_3 as the Te source. The other main change to the reaction scheme is the introduction of $NaBH_4$, which acts as a reducing agent generating Te^{2-} in solution. The CdTe QDs are prepared similarly to CdS and CdSe QDs, by using microwave-induced heating of a solution of stabilizer (cysteine or penicillamine), $Cd(ClO_4)_2{\cdot}6H_2O$, Na_2TeO_3 and $NaBH_4$. The typical experimental settings for the preparation of all Cd-based chiral QDs are also summarized in **Table 1**. In contrast to the cysteine-coated CdS and CdSe, it is possible to prepare only strongly intrinsically emitting, highly stable, cysteine-stabilized CdTe nanocrystals in water using this synthetic route. However, as we have previously reported, the chirality of QDs is due in part to the presence of chiral surface defects[12,13]. Therefore, the cysteine-coated intrinsically emitting dots demonstrate only very weak CD signals. Meanwhile, penicillamine-coated CdTe dots show weak defect and intrinsic emission, but they give a quite strong CD response.

CdS nanotetrapods are prepared by refluxing an aqueous solution of cadmium chloride with thioacetamide in the presence of penicillamine stabilizer for 2 h (**Fig. 2**). This method enables us

TABLE 1 | Typical experimental settings for the preparation of Cd-based chiral QDs using microwave heating.

Chiral QDs	CdS	CdSe	CdTe
Reagents	$Cd(ClO_4)_2{\cdot}xH_2O$ CH_3CSNH_2 $HSC_xH_yNH_2COOH$ NaOH	$Cd(ClO_4)_2{\cdot}xH_2O$ Na_2SeSO_3 $HSC_xH_yNH_2COOH$ NaOH	$Cd(ClO_4)_2{\cdot}xH_2O$ Na_2TeO_3/ $NaBH_4$ $HSC_xH_yNH_2COOH$ NaOH
Conditions of microwave synthesis	70 s at 850 W	70 s at 850 W	70 s at 850 W
UV-visible and PL spectra	345 nm; Abs. 1.1 492 nm; QY 12.8%	420 nm; Abs. 0.20 379 nm; Abs. 0.27 434 nm; QY 13.6%	505 nm; Abs. 0.37 555 nm;
CD spectra	208 ± 2 nm 250 ± 3 nm 299 ± 1 nm 318 ± 1 nm 340 ± 2 nm	220 ± 2 nm 255 ± 3 nm 300 ± 1 nm 360 ± 1 nm 420 ± 2 nm	220 ± 2 nm 255 ± 3 nm 300 ± 1 nm 505 ± 2 nm

Note: All experimental data given are for D-penicillamine–stabilized QDs. Abs, absorbance; QY, quantum yield.

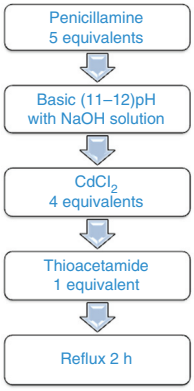

Figure 2 | General schematic for the production of CdS nanotetrapods by heating under reflux in an aqueous solution.

to produce quantum nanostructures with both a 3D tetrapodal morphology and optical activity in the band-edge region. In addition, it should be mentioned that, similarly to the chiral QDs discussed above, these nanostructures possess broad defect luminescence. In addition, the same approach can be used to prepare doped (e.g., Mn^{2+}, Cu^{2+} or Pb^{2+}) tetrapodal nanostructures that enable the control of their optical properties. Typical experimental settings for the preparation of CdS-based chiral nanotetrapods are presented in **Table 2**.

Finally, we also offer an alternative synthetic approach for the preparation of chiral CdS QDs. In this case, CdS is initially prepared from cadmium oxide (CdO), sulfur and oleic acid in 1-octadecene (ODE) using a traditional hot injection technique[40]. Oleic acid is a surfactant that stabilizes the CdS nanocrystals and the cationic precursors, and its amount highly affects the kinetics of the reaction. After degassing, the solution is heated to 315 °C to allow the formation of cadmium oleate, during which the solution undergoes a color change, from red to clear. Elemental sulfur dissolved in ODE is then added by hot injection at the desired temperature and growth proceeds for 10 min, after which the flask is allowed to cool down. The initial amount of ODE, hot injection temperature and growth time have to be monitored to control the size of the final QDs. The reaction time is proportional to the final size of the QDs; if the injection temperature is increased, then the size of the QDs will also increase. The particles are then precipitated, washed and centrifuged with acetone several times and stocked in toluene or chloroform. Next, we perform the phase transfer and induction of chirality in CdS QDs using a previously reported procedure[41], which has been modified by us. In this case, a basic L- or D-penicillamine solution in a small amount of methanol and water is stirred together with the above CdS QD stock solution for 3 h. Next, a large amount of water is added to promote the transfer into water phase and solubilization of QDs. After the phase-transfer procedure, the CD spectra of the penicillamine-capped CdS QDs show signals in the intrinsic absorbing region, demonstrating that chiral modification of the QDs has taken place. L- and D-penicillamine samples produce corresponding mirror-image CD spectra, as expected from nanoparticles modified by each enantiomer (ANTICIPATED RESULTS). A similar procedure involving the combination of the hot injection and phase-transfer techniques can also be used for the preparation of chiral Mn–doped ZnS QDs. This is presented at the very end of our protocol to demonstrate the potential universality of this approach.

TABLE 2 | Typical experimental settings for the preparation of CdS-based chiral nanotetrapods

Chiral QDs	CdS tetrapods	Mn-doped CdS tetrapods
Reagents	Ultrapure water (H_2O, via Milli-Q); Cadmium chloride hydrate ($CdCl_2 \cdot xH_2O$); Penicillamine ($(CH_3)_2C(SH)CH(NH_2)CO_2H$, D- and L-enantiomers) Thioacetamide (CH_3CSNH_2) NaOH Argon gas BOC	Ultrapure water (H_2O, via Milli-Q degassed before use) Cadmium chloride hydrate ($CdCl_2 \cdot xH_2O$) Manganese Nitrate ($Mn(NO_3)_2$) Penicillamine ($(CH_3)_2C(SH)CH(NH_2)CO_2H$, D- and L-enantiomers Thioacetamide (CH_3CSNH_2) NaOH Argon gas BOC
Conditions of synthesis	2-h reflux, evaporation under reduced pressure and passing through ultracentrifuge filters	Degassed water was used for the preparation of all the solutions as well as for the reaction itself which involved 2-h reflux synthesis under argon, evaporation under reduced pressure and passing through ultracentrifuge filters
UV-visible and PL spectra	UV-visible strong absorbance band at ~400 nm. PL broad emission, large Stokes' shift	UV-visible strong absorbance band at ~400 nm. PL broad emission, large Stokes' shift. Increasing levels of manganese doping led to a red shift in the maximum emission of the spectrum
CD spectra	CD spectra: the use of enantiopure stabilizers corresponds to the generation of optical signals in the band-edge region. The use of the opposite enantiomer generated a mirror image in the spectrum	CD spectra: the use of enantiopure stabilizers corresponds to the generation of optical signals in the band-edge region. The use of the opposite enantiomer generated a mirror image in the spectrum. Increasing levels of manganese dopant reduce the intensity of the optical signal

MATERIALS

REAGENTS

- Cadmium perchlorate hydrate, $Cd(ClO_4)_2{\cdot}xH_2O$ (x = ~6) (Sigma-Aldrich, cat. no. 401374)
- Cadmium oxide (CdO; ≥ 99.99%, cat. no. 202894, Sigma-Aldrich) **! CAUTION** CdO causes acute toxicity, and it is hazardous to health. Work in a fume hood.
- Cadmium chloride hydrate ($CdCl_2{\cdot}xH_2O$; 98%, Sigma-Aldrich, cat. no. 208299) **! CAUTION** $CdCl_2{\cdot}xH_2O$ causes acute toxicity, and it is hazardous to health. Work in a fume hood.
- Thioacetamide (CH_3CSNH_2; ACS reagent, for the precipitation (of heavy metals), ≥99.0%) (Sigma-Aldrich, cat. no. 88450) **! CAUTION** CH_3CSNH_2 causes acute toxicity and severe respiratory irritation).
- Na_2SO_3 (≥98%; Sigma-Aldrich, cat. no. S0505)
- Elemental Se (<4 mm, ≥99.99% trace metals basis; Sigma-Aldrich, cat. no. 209643)
- Elemental sulfur (powder, 99.98%, Sigma-Aldrich, cat. no. 744255)
- Sodium tellurite, Na_2TeO_3 (100 mesh, 99%; Sigma-Aldrich, cat. no. 400688)
- $NaBH_4$ (granular, 99.99% trace metals basis; Sigma-Aldrich, cat. no. 480886)
- Zinc chloride ($ZnCl_2$; anhydrous, free-flowing, Redi-Dri, reagent grade, ≥98% Sigma-Aldrich, cat. no. 793523)
- Manganese chloride ($MnCl_2$; powder and chunks, ≥99% trace metals basis; Sigma-Aldrich, cat. no. 244589)
- Manganese(II) nitrate hydrate ($Mn(NO_3)_2{\cdot}H_2O$, 98%; Sigma-Aldrich, cat. no. 288640)
- D-Cysteine (≥99% redox titration; RT, Sigma-Aldrich, cat. no. 30095)
- L-Cysteine (BioUltra, ≥98.5% (RT); Sigma-Aldrich, cat. no. 30089)
- D-Penicillamine (98-101%; Sigma-Aldrich, cat. no. P4875)
- L-Penicillamine (99%, Sigma-Aldrich, cat. no. 196312)
- Sodium hydroxide (NaOH; 97+% pellets; Fisher Scientific, cat. no. S/4880/60 **! CAUTION** NaOH is corrosive).
- Saturated $NaOH_{(aq)}$ (50% wt/wt) solution in Millipore ultrapure water should be prepared in advance, and it can be stored at ambient conditions for 1 month **! CAUTION** Saturated $NaOH_{(aq)}$ is corrosive.
- Dibenzylamine $((C_6H_5CH_2)_2NH$, 97%; Sigma- Aldrich, cat. no. D34108)
- Oleylamine ($C_{18}H_{37}N$, technical grade, 70% wt/wt; Sigma-Aldrich, cat. no. O7805)
- Chloroform ($CHCl_3$; CHROMASOLV Plusx, for HPLC, ≥99.9%, contains amylenes as stabilizer; Sigma-Aldrich, cat. no. 65049)
- Ethanol (C_2H_5OH; Fluka, CAS no. 67-17-5, ≥99.8%; HPLC grade, cat. no. 25894)
- Methanol (CH_3OH; CAS no. 67-56-1, ≥99.9%; HPLC grade, cat. no. 34860)
- Hydrochloric acid (HCl; CAS no. 7647-01-0, 37%, cat. no. 320331)
- Oleic acid ($C_{18}H_{34}O_2$; ≥99%, Sigma-Aldrich, cat. no. O1008)
- 1-Octadecene ($C_{18}H_{36}$; 90%, Sigma-Aldrich, cat. no. O806)
- Ultrapure Millipore water (H_2O, via Milli-Q)
- Millipore water was deoxygenated (where necessary) by heating it to boiling, and then by cooling it down under argon to room temperature (20–23 °C)

EQUIPMENT

- Round-bottomed flasks (RBFs), 100 ml
- Three-necked RBFs, 250 ml
- Aluminum heating blocks for 100- and 250-ml RBFs
- Argon cylinder (BOC)
- An argon bubbler
- Rubber septa
- Liebig reflux condenser
- 2× Quick-fit stopper
- Quick-fit gas bubbler with tap
- Quick-fit tap
- Disposable syringe, 5 ml
- Stainless steel needle, ≥10 cm
- D13 B24 to B14 connectors
- B-14 Liebig condenser
- 40-kDa ultracentrifuge nanofilters (Pall Nanosep; Sigma-Aldrich, cat. no. Z722111)
- Variable-speed ultracentrifuge (Hermle Z 233 M-2)
- Centrifuge (Hettich Universal 32)
- Centrifuge (Eppendorf centrifuge, model 5410)
- Magnetic stirring hot plate (IKA)
- Shaker (IKA Ks130)
- HANNA HI 991001 temperature and pH meter
- Microwave. CEM Star System 6
- Rotary evaporator (Heidolph Laborota 4002)
- FEI Titan transmission electron microscope
- Jeol Jem 2100 transmission electron microscope
- UV-visible Cary 500 spectrometer
- Photoluminescence spectrometer (Cary Eclipse)
- CD spectrometer a JASCO J-810
- Electron microscopy analysis was performed using a JEOL JEM-2100 or a FEI Titan electron microscope
- Formvar grids using a 200-KV accelerating voltage and a beam current of 105 μA
- EDX analysis was conducted using a MIRA TESCAN SEM system

PROCEDURE

Preparation of chiral CdS, CdSe and CdTe QDs using microwave heating

▲ **CRITICAL** **Table 3** presents an overview of the procedures for the synthesis of chiral QDs. Step 1 should be used to produce broad defect–emitting chiral Cd–based II–VI QDs such as CdS, CdSe and CdTe in an aqueous solution. If it is necessary to prepare chiral broad defect–emitting CdS nanotetrapods or Mn-doped CdS nanotetrapods in water, the approach in Step 2 is used. Step 3 should be used to prepare narrow-emitting chiral CdS or Mn-doped ZnS QDs by a combination of hot injection and phase-transfer techniques.

1| In this section (see also **Table 3**), chiral QDs are prepared in water using a microwave heating technique. There are six options, as summarized below:

1A	Penicillamine-stabilized CdS QDs
1B	Cysteine-stabilized CdS QDs
1C	Penicillamine-stabilized CdSe QDs
1D	Cysteine-stabilized CdSe QDs
1E	Penicillamine-stabilized CdTe QDs
1F	Cysteine-stabilized CdTe QDs

TABLE 3 | Summary of the procedures for the synthesis of chiral QDs.

	Specific feature of QDs produced by this approach	Advantages	Limitations
Preparation of chiral CdS, CdSe and CdTe QDs using microwave heating (Step 1)	QDs can be produced in water, and they demonstrate broad defect luminescence emission; optical activity and CD properties of QDs are related to chiral defects	• Very fast (e.g., 70 s) and convenient synthesis • It can be potentially performed in microwave continuous-flow reactors, enabling scale-up	• Very precise control of microwave power and time is necessary to get reproducible results • Limited control over shape and size of QDs • Yields are quite low • The procedure still requires to leave the QDs to mature for at least 24 h • No spectroscopic control of the process during the synthesis
Preparation of chiral CdS nanotetrapods by heating under reflux (Step 2)	This approach enables the production of chiral tetrapod-like quantum nanostructures in water; these nanostructures demonstrate broad defect luminescent emission; optical activity and CD properties of QDs are related to chiral defects	• Provides good control over size and shape of QDs (by controlling temperature and heating time) • Relatively easy to monitor by spectroscopic techniques (taking probes at certain time during the synthesis)	• Requires longer preparation times (e.g., several hours of heating) • Yields are quite low
Preparation of chiral CdS and Mn-doped ZnS QDs using a combination of hot injection and phase transfer techniques (Step 3)	QDs can be produced in organic solvents; QDs have a narrow emission; CD is related by the hybridization of the chiral ligand's HOMOs with molecular orbitals of the II–VI species at QD surface	• Good yields • High quantum efficiencies of QDs • Good control of the size and shape of QDs	• The synthesis requires high temperatures (up to 315 °C) • Involve large amounts of sulfur-generating poisonous gases • QDs partially lose quantum efficiency during the phase-transfer process • Stability of the QDs in solution can be worsened after the phase-transfer process

(A) Preparation and purification of chiral penicillamine-stabilized CdS QDs ● TIMING 72 h

(i) Using Millipore water, freshly prepare 1×10^{-2} M aqueous stock solutions of D-penicillamine, L-penicillamine, *Rac*-penicillamine, $Cd(ClO_4)_2$ and CH_3CSNH_2.
? TROUBLESHOOTING

(ii) Ensure that three drops of saturated $NaOH_{(aq)}$ (50.0% wt/wt) solution are added to the penicillamine stock solutions and that they are well mixed.
▲ **CRITICAL STEP** If the pH is too low, then the QDs will not form.

(iii) Add 45 ml of Millipore water to a microwave vessel.

(iv) Add 4 ml of a (D- or L-) basic aqueous 1×10^{-2} M penicillamine stabilizer stock solution, making a solution containing 2×10^{-5} m stabilizer. If racemic dots are required, then 2 ml of both the D- and L-stock solutions (50:50) are used instead. Cap the microwave vessel and shake the contents.
? TROUBLESHOOTING

(v) Adjust the pH to 12 by the dropwise addition of saturated $NaOH_{(aq)}$ (50.0% wt/wt) solution. Cap the microwave vessel and shake the contents.
▲ **CRITICAL STEP** If the pH is too low (<12), then QDs will not form.
? TROUBLESHOOTING

(vi) Add 8 ml of the 1×10^{-2} M $Cd(ClO_4)_2$ stock solution. Cap the microwave vessel and shake the contents.
? TROUBLESHOOTING

(vii) Add 2 ml of the 1×10^{-2} M of thioacetamide (CH_3CSNH_2) stock solution. Cap the microwave vessel and shake the contents vigorously.
? TROUBLESHOOTING
(viii) Connect the condenser to the microwave vessel and place it in a CEM Star System 6 instrument.
(ix) Microwave-heat the mixture for 70 s at 850 W.
(x) Use heat-protective gloves to remove the microwave vessel from the microwave.
(xi) Transfer the resulting solution into 100 ml of RBF and wrap it in aluminum foil.
■ **PAUSE POINT** The solution is then matured in a cool, dark location for at least 24 h (up to 3 d).
(xii) After the allocated time passes, remove the foil and connect the RBF to a rotary evaporator. Reduce the volume of the colloid to ~5 ml.
(xiii) Add propan-2-ol dropwise using a glass pipette until a milky precipitate is formed.
▲ **CRITICAL STEP** Once the precipitate is formed, swirl the RBF and the precipitate will probably 're-dissolve'. If this takes place, then simply add more drops of propan-2-ol, swirling until the milky precipitate remains. However, too much propan-2-ol might also 're-dissolve' the particles, and thus it should be added carefully and dropwise.
? TROUBLESHOOTING
(xiv) Transfer the contents of the RBF to 50-ml centrifuge tubes, and centrifuge the content at 1,000*g* for 15 min at room temperature. Discard the supernatant.
(xv) Add 5 ml of Millipore water to re-disperse the precipitate.
(xvi) Perform Step 1A(xiii–xv) two more times.
(xvii) Re-disperse the purified particles (the expected yield is 0.8 mg) in 40 ml of Millipore water, and then transfer them to a clean dry RBF.

(B) Preparation and purification of chiral cysteine-stabilized CdS QDs ● TIMING 72 h
(i) Perform Step1A with the following modifications. In the case of cysteine-stabilized CdS dots, use 4 ml of $Cd(ClO_4)_2$, 2 ml of D- or L- cysteine and 2 ml of CH_3CSNH_2 stock solutions while precisely following the procedure described for the penicillamine CdS dots.

(C) Preparation and purification of chiral penicillamine-stabilized CdSe QDs ● TIMING 72 h
(i) For the preparation of penicillamine-stabilized chiral CdSe QDs, thioacetamide is replaced with the water-soluble Se source Na_2SeSO_3. A degassed 1×10^{-2} M stock solution of Na_2SeSO_3 must therefore be prepared before the CdSe QDs can be made. To do this, follow the steps in **Box 1**.
▲ **CRITICAL STEP** As the preparation of Na_2SeSO_3 requires an overnight reflux of Na_2SO_3 and elemental Se in degassed Millipore water, this preparation should be done on the day before the CdSe synthesis is to take place.
(ii) Add 45 ml of degassed Millipore to a 250-ml three-necked RBF, and bubble argon through for 15 min.
(iii) Add 2 ml of 1×10^{-2} M D- or L- (or 1 ml of D- and L- for the *Rac*-dots) penicillamine stock solution under constant stirring, and under argon flow. Adjust the pH to 12 using a saturated $NaOH_{(aq)}$ (50.0% wt/wt) solution.
(iv) Add 2 ml of degassed 1×10^{-2} M $Cd(ClO_4)_2$ stock solution under constant stirring.
(v) Add by syringe 2 ml of degassed 1×10^{-2} M Na_2SeSO_3 stock solution under constant stirring.
(vi) Remove the magnetic stirrer and argon bubbler and put stoppers in the RBF.
(vii) Transfer the contents of the RBF to the microwave vessel as described for CdS above.
(viii) After microwave irradiation, remove the microwave vessel from the microwave, transfer the contents into an RBF, put stoppers in and cover the RBF in aluminum foil, as described for CdS above. The clear yellow-orange CdSe colloid is matured and worked up exactly as described earlier for CdS (i.e., follow Step 1A(xi–xvi)). The expected yield is 0.9 mg.

(D) Preparation and purification of chiral cysteine-stabilized CdSe QDs ● TIMING 72 h
(i) Follow Step 1C with the following modifications: in the case of cysteine-stabilized QDs, use 8 ml of the degassed 1×10^{-2} M $Cd(ClO_4)_2$ stock solution, 8ml of the cysteine and 2 ml of the Na_2SeSO_3 stock solutions.

(E) Preparation and purification of chiral penicillamine-stabilized CdTe QDs ● TIMING 72 h
▲ **CRITICAL** The preparation of these dots is engineered to resemble the CdS preparation as closely as possible. However, in this case, Na_2TeO_3 is used in place of CH_3CSNH_2 as a source of the Te^{2-} anion.
(i) Add in sequence 2 ml of the 1×10^{-2} M D- or L- (or 1 ml of both D- and L- for *Rac*-dots) penicillamine stock solution, 4 ml of the 1×10^{-2} M $Cd(ClO_4)_2$ and 2 ml of a 1×10^{-2} M Na_2TeO_3 stock solution to 45 ml of Millipore water in a 250-ml RBF, and then adjust the pH to 12 as described previously for the preparation of CdS.
(ii) Simultaneously, prepare a 1×10^{-1} M solution of $NaBH_4$ in Millipore water.
(iii) Add 2 ml of the $NaBH_4$ stock solution to the RBF. The flask is closed and shaken well. The contents of the flask are then transferred to the microwave and irradiated as described previously.
(iv) The dots are stored and worked up as described previously. The expected yield is 1 mg.

Box 1 | Procedure for the preparation of Na_2SeSO_3 ● TIMING 24 h

Na_2SeSO_3 is required for the preparation of chiral penicillamine- and cysteine-stabilized CdSe QDs. It is crucial that the resulting clear colorless solution is used immediately and is removed using a needle and syringe under constant argon flow.

MATERIALS

Reagents

- Na_2SO_3 (≥98%, Sigma-Aldrich, cat. no. S0505)
- Elemental (Se, pellets, <4 mm, ≥99.99% trace metals basis; Sigma-Aldrich, cat. no. 209643)
- Millipore water was deoxygenated/degassed (where necessary) by heating it to boiling and then cooling down under argon to room temperature.

Equipment

250-ml three-necked RBF
Argon cylinder (BOC)
Argon bubbler
2× rubber septa
Liebig reflux condenser
2× Quick-fit stopper
Quick-fit gas bubbler with tap
Quick-fit tap
5-ml disposable syringe
≥10 cm stainless steel needle
Magnetic stirring hot plate (IKA)

Procedure

1. Fit a 250-ml three-necked RBF with the reflux condenser on the central neck and an argon bubbler (with tap) on one of the ancillary necks. The third neck remains open.
2. Add 50 ml of degassed Millipore water under a constant argon flow to RBF. Add 0.118 g of Na_2SO_3 and wait for total dissolution.
3. Add 0.0395 g of (water insoluble) elemental Se under constant stirring.
4. Close the open neck with a rubber septum. Continue to bubble argon through the water for an additional 10 min before closing the quick-fit tap.

▲ CRITICAL STEP Stir the mixture extremely vigorously to ensure that the water-insoluble Se does not simply stick to the sides of the RBF.

5. Connect the argon quick-fit tap to the condenser and bring the contents to reflux under a constant flow of argon.
6. Once reflux is reached, stop the argon flow. Keep the quick-fit tap open and allow the mixture to reflux overnight.
7. On the next day, check the solution to ensure that no black Se powder remains.

▲ CRITICAL STEP If any black Se powder can be seen at the bottom of the RBF, then the solution cannot be used, and a fresh batch must be prepared.

? TROUBLESHOOTING

8. Before removing the heat source, flow argon through the quick-fit tap fitted to the top of the condenser. This ensures that no air enters the system as it cools.

(F) Preparation and purification of chiral cysteine-stabilized CdTe QDs ● TIMING 72 h

(i) Perform Step 1E with the following modifications: use 8 ml of the $Cd(ClO_4)_2$ and 4 ml of the cysteine stock solutions.

Preparation of chiral CdS nanotetrapods by heating under reflux

2| In this section, chiral CdS and Mn-doped CdS nanotetrapods are prepared in water using heating under reflux technique. Perform Step 2A for the preparation of penicillamine-stabilized CdS nanotetrapods and Step 2B for the preparation of penicillamine-stabilized Mn-doped CdS nanotetrapods.

(A) Preparation of chiral pristine penicillamine-stabilized CdS nanotetrapods ● TIMING 24 h

(i) Add 40 ml of water into a 100-ml RBF.
(ii) To this add penicillamine solution (10 ml 0.01M) (either D-penicillamine, L-penicillamine or a 50:50 mixture of D-and L-penicillamine for the *Rac*-penicillamine stabilizer) in water.
(iii) Add NaOH in water (0.4ml, 2 M) to increase the pH to ~11–12.

▲ CRITICAL STEP The addition of NaOH to increase the pH of the solution is needed to ensure particle formation.

(iv) Add $CdCl_2$ solution (8 ml, 0.01 M) in water.
(v) Add thioacetamide (2 ml, 0.01 M) in water.

(vi) Heat the solution under reflux at 130 °C for 2 h with stirring using a stirrer and hot plate.
? TROUBLESHOOTING
(vii) Stop heating and remove the flask with the solution from the hot plate.
(viii) Wrap the samples in aluminum foil.
■ PAUSE POINT These samples can be stored overnight at ambient temperature.
(ix) Reduce the volume of solution via evaporation using a rotary evaporator at 50 °C under reduced pressure to ~1 ml.
? TROUBLESHOOTING

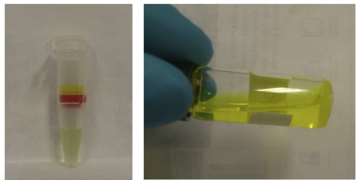

Figure 3 | Images relevant to troubleshooting. Left, Step 2A(xi); fluid no longer flows through membrane filters; right, Step 2A(xi), continued precipitation of nanotetrapods while in storage.

(x) Transfer the entire reduced solution (~1 ml) to an Eppendorf centrifuge tube, and then centrifuge it at 1,800*g* for 1–3 min at room temperature to remove larger aggregated material.
(xi) Pipette the mixture in 400-µl aliquots into a 30-kDa ultracentrifuge filter, and then the mixture is passed through by centrifugation at 1,780*g* for 15 min at room temperature. Millipore water should be passed though the filter up to four times, and the product should then be re-dispersed in water and transferred to an appropriate vessel. The expected yield is 1.1 mg (**Fig. 3**).
? TROUBLESHOOTING
■ PAUSE POINT. Store the product, preferably under refrigerated conditions. We would expect it to be stable for at least 1 month.
(xii) Analyze the CdS nanotetrapod samples using UV-visible, PL and CD spectroscopy. Samples can also be investigated using TEM analysis.

(B) Preparation of penicillamine-stabilized, Mn-doped CdS nanotetrapods ● TIMING 24 h

(i) Follow Step 2A with the following modifications: in the case of penicillamine-stabilized, Mn-doped CdS nanotetrapods, use Millipore water that has been degassed under argon for the reactions and for the preparation of the reagent solutions. Controlled amounts of $Mn(NO_3)_2$ solution (1 ml, 0.01 M) in degassed water should be added to the reaction mixture before it is heated under reflux. The expected yield is 0.8 mg.

Preparation of chiral CdS and Mn-doped ZnS QDs using a combination of hot injection and phase-transfer techniques

3| In this section, initially nonchiral CdS and Mn-doped ZnS QDs are prepared in organic medium using the hot injection technique, and then chirality is introduced to the QDs by their phase transfer into water in the presence of water-soluble chiral-stabilizing ligands. Perform Step 3A for the preparation of penicillamine-stabilized CdS QDs and Step 3B for the preparation of penicillamine-stabilized, Mn-doped ZnS QDs.

(A) Preparation of chiral penicillamine CdS QDs by a combination of hot injection and phase-transfer techniques ● TIMING 11 h

(i) *Preparation and purification of CdS QDs in organic medium*. Mix CdO (12.8 mg), oleic acid (93 mg) and ODE (3.9 g) in a 100-ml three-necked RBF fitted with a thermometer and a septum, and degas the mixture by placing it under vacuum for 30 min and then by flashing it with argon gas.
(ii) Heat the solution to 315 °C using a hot plate and aluminum heating block to form Cd oleate, a colorless solution.
(iii) In another flask, dissolve sulfur (16 mg) in degassed ODE (10 ml) by heating it to 200 °C for 15 min and then by cooling it to 100 °C.
(iv) Inject 1 ml of this sulfur solution into the hot (100 °C) Cd oleate solution using a syringe, and then allow the reaction to proceed for 15 min.
▲ CRITICAL STEP The temperature of the Cd oleate solution defines the final size of the QDs. To achieve smaller sizes, decrease the temperature of the Cd oleate solutions.
(v) Remove the solution from heat and quench it with degassed acetone (20 ml).
(vi) Wash the QDs with acetone and centrifuge three times (1,370*g*, 10 min) at room temperature.
(vii) Disperse the QDs in toluene (20 ml).
■ PAUSE POINT The QD solution may be stored in the fridge for up to 3 months.
(viii) Analyze the product using UV-visible and PL spectroscopy.
(ix) *Preparation and purification of water-soluble penicillamine-capped CdS QDs*. Dissolve D- or L-penicillamine (30 mg) in Millipore water (150 µl) and methanol (0.5 ml).
(x) Adjust the pH of the solution to 10 using a stock NaOH solution (0.5 M).
(xi) Combine this solution with 2.5 ml of the CdS in toluene solution and stir it for 3 h to transfer the QDs to the methanol layer.

(xii) Add Millipore water (5 ml) to the solution and stir it for a further 30 min.
(xiii) Collect the aqueous layer and precipitate the QDs by adding acetone.
(xiv) Centrifuge the solution (1,370*g*, 10 min) at room temperature and re-disperse the resulting QD pellet in Millipore water (5 ml).
(xv) Adjust the pH of the solution to 10 using a solution of NaOH (0.5 M).
(xvi) Repeat Step 3A(viii–x) using L-penicillamine instead of D-penicillamine to produce the corresponding L-penicillaminestabilized QDs.
(xvii) Analyze your product using UV-visible, CD and PL spectroscopy. The expected yield is 8 mg.

(B) Preparation of chiral penicillamine-stabilized, Mn-doped ZnS QDs ● TIMING 77 h

(i) *Preparation and purification of Mn-doped ZnS QDs in organic medium*. Add $ZnCl_2$ (0.6 g) and $MnCl_2$ (0.02 g) and dibenzylamine (54 ml) to a 100-ml three-necked RBF.
(ii) Heat the mixture under vacuum at 120 °C for 2 h. We call this pot 1.
▲ **CRITICAL STEP** It is important that the chemicals remain under vacuum while heating, as both $ZnCl_2$ and $MnCl_2$ are highly hydroscopic; it is essential to remove as much water as possible to improve the quality of the QDs.
(iii) While pot 1 is heating under vacuum, add $ZnCl_2$ (0.8 g) and dibenzylamine (10 ml) to a 25-ml RBF.
? TROUBLESHOOTING
(iv) Heat also the 25-ml RBF at 120 °C for ≈ 2 h under vacuum. We call this pot 2.
(v) Once pot 1 has been heated for 2 h under vacuum, allow the solution to cool to 50 °C.
(vi) Flash the flask with argon and then add sulfur (0.6 g) to pot 1.
(vii) Heat the solution under reflux at 260 °C for 15 min.
(viii) After 15 min, allow the solution to cool to 160 °C.
(ix) Flash pot 2 with argon and add 5 ml of solution from pot 2 to pot 1.
(x) Heat pot 1 under reflux to 260 °C under argon and maintain it at this temperature for a further 15 min.
(xi) Cool the solution to ~120 °C.
(xii) Add ethanol (40 ml) to the solution to precipitate out the QDs.
(xiii) Divide the solution between four 50-ml centrifuge tubes, and add ~20 ml of additional ethanol to each centrifuge tube.
(xiv) Agitate the tubes to disperse the QDs, and then centrifuge at 1,370*g* for 10 min at room temperature.
(xv) Remove the supernatant from each tube and wash the QDs with ethanol, and then centrifuge three more times (1,370*g*, 10 min, room temperature) or until the yellow color from the supernatant is no longer present.
(xvi) Dissolve the washed QDs in $CHCl_3$ (25 ml) and add 0.1 ml of oleylamine for improved stability.
■ **PAUSE POINT** This QD solution may be stored in the refrigerator (4 °C) for up to 6 months.
(xvii) Characterize the QDs by TEM, UV-visible and PL spectroscopy.
(xviii) *Preparation of aqueous D/L-penicillamine–stabilized, Mn-doped ZnS using phase-transfer methods*. Add methanol (0.5 ml) to an Eppendorf tube containing the previously prepared QD solution (0.5 ml).
(xix) Centrifuge the solution at 1,780*g* for 10 min at room temperature and remove the supernatant.
(xx) Re-disperse the QD pellet in 1 ml of $CHCl_3$.
(xxi) In a separate Eppendorf tube, combine methanol (600 µl), HCl (20 µl) and L-penicillamine (20 mg).
? TROUBLESHOOTING
(xxii) Add 50 µl of this solution to the QD/$CHCl_3$ solution.
(xxiii) Shake the solution for 24 h or until the solution becomes cloudy.
? TROUBLESHOOTING
(xxiv) Centrifuge the solution at 1,780*g* for 10 min at room temperature and remove the supernatant.
? TROUBLESHOOTING
(xxv) Wash the QDs with 10 ml of methanol twice, and centrifuge it at 1,780*g* for 1–3 min at room temperature to remove the methanol from the QDs.
(xxvi) Re-disperse the resulting QDs in Millipore water (10 ml) and adjust the pH to 11–12 using a 0.5 M solution of NaOH to improve aqueous stability.
▲ **CRITICAL STEP** It is important to ensure that the pH of the solution is at least 11; otherwise, the QDs may aggregate and precipitate out of solution.
(xxvii) Repeat Step 3B(xviii–xxvi) using D-penicillamine instead of L-penicillamine to create D-penicillamine–stabilized QDs.
■ **PAUSE POINT** This QD solution may be stored in the refrigerator (4 °C) for up to 2 months.
(xxviii) Characterize the QDs using UV-visible and PL spectroscopy.
(xxix) *Preparation of chiral Mn-doped ZnS*. Add L- or D- or *Rac*-penicillamine (50 mg) to the L- or D- or Rac-penicillamine–stabilized QD solution, respectively, and then heat the resulting solution at 50 °C for 48 h.
■ **PAUSE POINT** This QD solution may be stored in the refrigerator (4 °C) for up to 2 months.
(xxx) Once 48 h has elapsed, allow the QDs to cool, and then characterize them using UV-visible, PL and CD spectroscopy. The expected yield is 0.2 g.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 4**.

TABLE 4 | Troubleshooting table.

Step	Problem	Possible reason	Solution
1A(i)	Low solubility of stabilizer	Insufficient addition of $NaOH_{(aq)}$	pH must be >11
1A(iv–vii)	Insolubility of $Cd(ClO_4)_2$ at high pH	Incorrect sequence of addition	Stabilizer stock solution must be added to the reaction solution before/ and never after Cd stock solution. Order of addition is stabilizer, $NaOH_{(aq)}$, Cd^{2+} and finally X^{2-}
1A(vii), before microwave step	pH is too low. Dots are not formed, or they are far below the expected concentration	Insufficient addition of $NaOH_{(aq)}$	pH must be >11
Box 1, step 7, Na_2SeSO_3 preparation (CdSe only)	Presence of unreacted Se	Insufficient reflux time Presence of oxide layer Insufficient concentration of Na_2SO_3	Reflux for 24 h Se should be kept in the glove box. The system should be properly degassed before starting reflux Ensure that greater than 1 molar ratio of Na_2SO_3 to Se is used at all times (i.e., there should be a slight excess of Na_2SO_3)
1A(xiii), washing step	Particles won't precipitate	Too much water Too much propan-2-ol	Use a rotary evaporator to reduce the water volume to ~5 ml Add propan-2-ol dropwise while continually swirling the flasks contents until a milky precipitate appears and stays
2A(vi)	Precipitate formation at the end of the reflux	Instability of nanoparticles owing to a lack of surface charge	Add more NaOH solution to deprotonate the ligand coating and allow stable dispersion
2A(ix)	Precipitate formed during evaporation	Aggregation owing to increased concentration	Add Millipore water with swirling to re-disperse, followed by centrifugation to remove persistent aggregates
2A(xi)	Liquid flow-through ultracentrifuge filter slows or stops completely (**Fig. 3**, left)	Clogging of filter pores with small particles or distortion of filter membrane owing to mechanical stress	Transfer the suspension to a fresh filter and try again
	Aggregation during storage (**Fig. 3**, right)	Aggregation owing to instabilities of the suspended nanoparticles	Agitate to re-disperse, and then centrifuge to remove persistent aggregates
3B(iii)	Solution solidifies	Not enough dibenzylamine to form the solution properly	Use a larger stirring bar and a 50-ml RBF
3B(xxi)	Penicillamine is not dissolving	Requires a higher concentration of HCl	Use 37% concentrated HCl, add an extra 20 µl if required
3B(xxiii)	Particles are not precipitating out	Needs more time	Continue to shake it until a precipitate develops
3B(xxiv)	QDs are not separating from solution	Dibenzylamine solution is too viscous still	Add more ethanol before centrifuging

● TIMING

Step 1A, preparation and purification of chiral penicillamine-stabilized CdS QDs: 72 h
Step 1B, preparation and purification of chiral cysteine-stabilized CdS QDs: 72 h
Step 1C, preparation and purification of chiral penicillamine-stabilized CdSe QDs: 72 h
Step 1D, preparation and purification of chiral cysteine-stabilized CdSe QDs: 72 h
Step 1E, preparation and purification of chiral penicillamine-stabilized CdTe QDs: 72 h
Step 1F, preparation and purification of chiral cysteine-stabilized CdTe QDs: 72 h
Step 2A, preparation of chiral pristine penicillamine-stabilized CdS nanotetrapods: 24 h
Step 2B, preparation of penicillamine-stabilized, Mn-doped CdS nanotetrapods: 24 h
Step 3A(i–viii), Preparation and purification of CdS QDs in organic medium: 7 h
Step 3A(ix–xvii), preparation and purification of water-soluble penicillamine-capped CdS QDs: 4 h
Step 3B(i–xvii), preparation and purification of Mn-doped ZnS QDs in organic medium: 3 h
Step 3B(xviii–xxviii), preparation of aqueous D/L-penicillamine-stabilized Mn-doped ZnS using phase-transfer methods: 25 h
Step 3B(xxix and xxx), preparation of chiral Mn-doped ZnS: 49 h
Box 1, procedure for the preparation of Na_2SeSO_3: 24 h

ANTICIPATED RESULTS

All the colloids produced should be clear with no sedimenting particles. UV-visible spectra of chiral penicillamine–stabilized CdS QDs (Step 1A) should show no scattering with clearly defined band edges at the appropriate areas (**Fig. 4**). The CdS samples should be colorless, but they should shine blue-white or green-white under UV light. CdSe will be yellow-orange, whereas the CdTe will be green, yellow or red depending on the stabilizer used.

CD spectra of D- and L-penicillamine–stabilized CdS QDs should produce a corresponding mirror image CD scans (**Fig. 4**), whereas the particles prepared with a *Rac* mixture should show only a weak signal. The CD spectra should be quite different from that of the free D- and L-penicillamine, which show, as expected, a near-symmetrical image with maxima/minima at 234 ± 2 nm. Meanwhile, the CD spectra of D- and L-penicillamine–stabilized CdS QDs should be more complex, with maxima/minima at 207 ± 3,252 ± 2,293 ± 3,320 ± 2 and 345 ± 2 nm (**Fig. 4**), wavelengths that are much longer than those at which the penicillamine ligands themselves absorb.

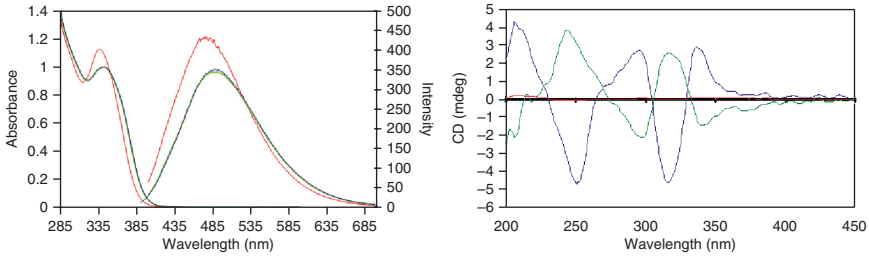

Figure 4 | Characterization of particles prepared as described in Step 1A. Left: UV-visible and emission (Ems) spectra of D-penicillamine CdS, (blue), absorbance (Abs) 345 nm, Ems 492 nm, L-penicillamine CdS, (green), Abs 345 nm, Ems 492 nm and *Rac*-penicillamine CdS (red). All PL spectra were excited at 360 nm. The higher quantum yield of the *Rac*-dots is clearly visible in the picture. Right, CD spectra of D-penicillamine (red), L-penicillamine (green), and *Rac*-penicillamine (blue)-modified CdS particles.

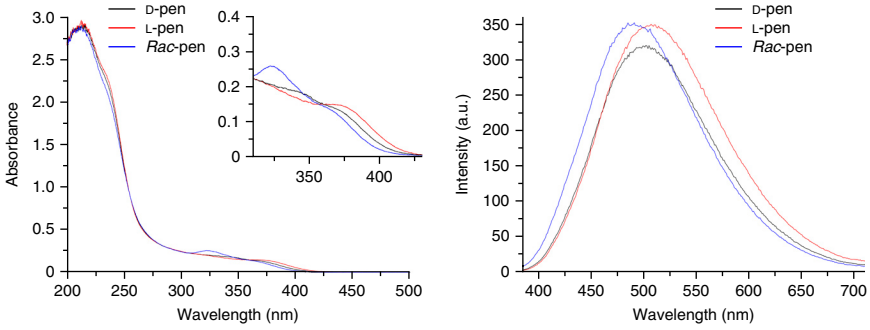

Figure 5 | UV-visible spectra of nanotetrapods prepared as described in Step 2A. Left, UV-visible spectra of penicillamine (pen)-stabilized CdS nanotetrapods. Inset, close-up of the band-edge region highlighting the difference between D-, L- and *Rac* samples. Right, PL spectra of penicillamine–stabilized CdS nanotetrapods. a.u., arbitrary units.

Examination of the CdS nanotetrapod samples by UV-visible spectroscopy (**Fig. 5**) should show a gradual increase in absorbance with decreasing wavelength. There is a maximum in absorbance around the 400-nm region, which corresponds to the band-edge absorbance of the quantum-confined nanoparticles. The maximum absorbance <300 nm can be identified

as coming from both further transition and absorbance in the near-UV region of the ligand sphere. PL spectra of the CdS nanotetrapods (Step 2A) should show a broad emission with a full-width at half maximum and Stokes' shift of >100 nm, both of which are indicative of defect luminescence (**Fig. 5**).

The CD spectra (**Fig. 6**) of the CdS tetrapod samples produced using enantiomerically pure ligands should show a series of CD signals. The bands for the D-penicillamine–stabilized nanotetrapods show approximate mirror images to the L-penicillamine–stabilized nanotetrapods. It may also be noted that most of these signals in the same region of the first band edge transition are far more red-shifted than pre-existing signals from the ligands. This indicates that these signals come from the QD itself either by an interaction with the ligand or by the distortion of the nanoparticle surface.

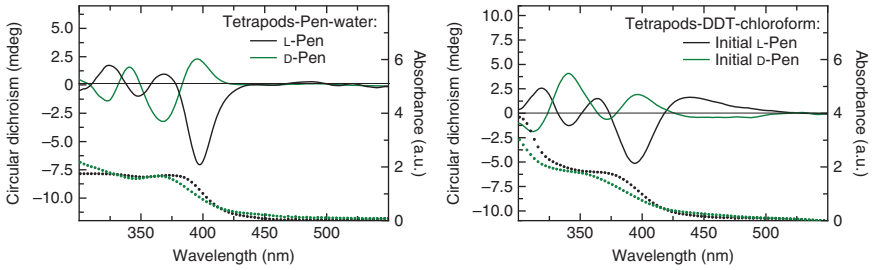

Figure 6 | CD spectra of nanotetrapods prepared as described in Step 2A. Left, CD spectra of penicillamine (pen)-stabilized CdS nanotetrapods. Right, CD spectra of penicillamine-stabilized CdS nanotetrapods after their phase transfer into chloroform and removal of chiral ligands using a nonchiral 1-dodecanethiol ligand (DDT). a.u, arbitrary units.

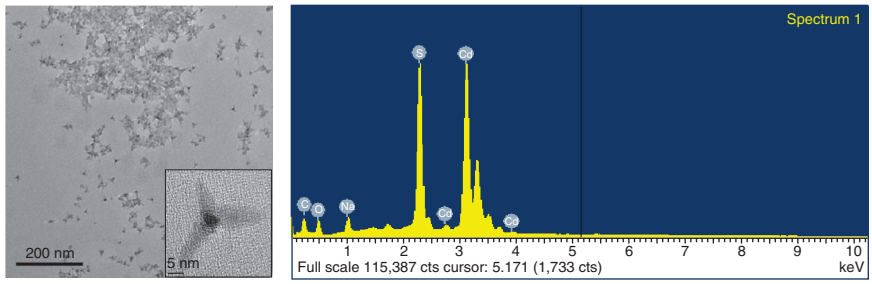

Figure 7 | TEM images of nanotetrapods prepared as described in Step 2A. Left, TEM images of L-penicillamine–stabilized CdS nanotetrapods, inset: high-resolution TEM image of an individual CdS nanotetrapod. Right, EDX spectra of D-penicillamine–stabilized CdS nanotetrapods.

In addition, it is possible to perform the phase transfer of chiral quantum tetrapods into organic phase (e.g., $CHCl_3$) using nonchiral dodecanethiol ligand. This process results in an organic solution of chiral tetrapods in $CHCl_3$, which should show an optical activity and chiral signals without having any chiral ligands around (**Fig. 6**). We must notice that the CD spectra (positions and intensities of the signals) of CdS tetrapods after the phase transfer are altered compared with those before. This is due to the removal of the initial chiral penicillamine ligands from the surface; in addition, the solvent used is also completely different ($CHCl_3$ instead of water). Fourier transform infrared spectroscopy analysis of the organic phase shows no trace of any chiral ligands present. This is a demonstration of the so-called 'chiral memory' effect, which was previously reported for chiral CdTe QDs by Kawai and colleagues[19]. This experiment shows that in this case the optical activity of thiol–capped QDs originates from distorted chiral QD surfaces (chiral defects), which can be transferred to a different phase and retained without any chiral ligands present.

TEM images (**Fig. 7**) of the samples show multiple tetrapodal nanostructures (three arms in the plane of the grid and one sticking up) across the sample. Examination of the EDX spectra (**Fig. 7**) of samples shows strong peaks for both cadmium and

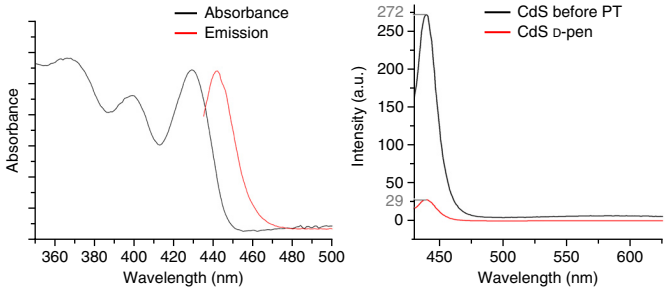

Figure 8 | Characterization of nanoparticles prepared as described in Step 3B. Left, UV-visible (black) and PL (red) spectra for the CdS QDs. Excitation wavelength for the PL spectra was 430 nm. Right, PL spectra for the CdS QDs before (black) and after (red) the phase-transfer (PT) process. The QDs were excited at 430 nm. a.u., arbitrary units.

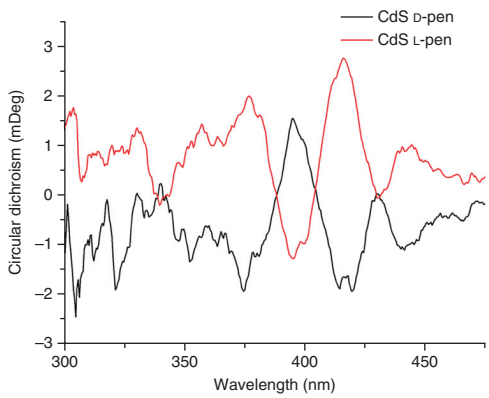

Figure 9 | CD spectra for the CdS QDs after the phase transfer (Step 3B).

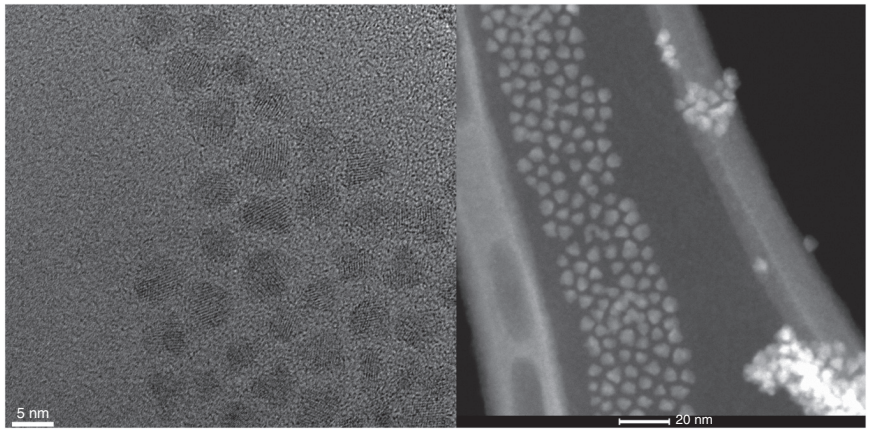

Figure 10 | Electron images of nanoparticles prepared as described in Step 3B. Right, scanning TEM image of ZnS:Mn QDs. Left, high-resolution TEM of the same QDs.

sulfur, indicating that it dominates the constitution of the nanotetrapods.

The UV-visible and PL spectra (**Fig. 8**) of CdS QDs, which are produced by the hot injection technique, show a maximum absorbance for the first exciton at 398 nm. Subsequent emission has a maximum at 413 nm. Slight emission may be seen between 450 nm and 700 nm, which is due to some defects in the CdS.

The QDs were then transferred into aqueous medium using D- and L-penicillamine to make them water soluble. Although the QDs can be seen to still be luminescent, there is a substantial drop in luminescence, which is commonly seen with phase-transfer methods (**Fig. 8**). This process is similar to the one recently reported for producing chiral CdSe–based QDs and quantum rods by transferring their nonchiral precursor QDs or rods to aqueous phase using L-and D-cysteines[21,22] .

The CD spectra (**Fig. 9**) for the D-penicillamine–stabilized CdS and the L-penicillamine CdS QDs show optical activity that corresponds to the excitonic peaks of the CdS QDs in the UV-visible spectra, which are located in the visible region, unlike free penicillamine. The D-penicillamine and L-penicillamine QD solutions yield mirror-image spectra of each other.

From the scanning TEM and high-resolution TEM images (**Fig. 10**), it can be seen that the QDs are highly crystalline with an average size of 5.3 nm.

The EDX spectra (**Fig. 11**) of the QDs show strong signals for both zinc and sulfur, with smaller signals for manganese, confirming the successful doping of ZnS QDs with manganese.

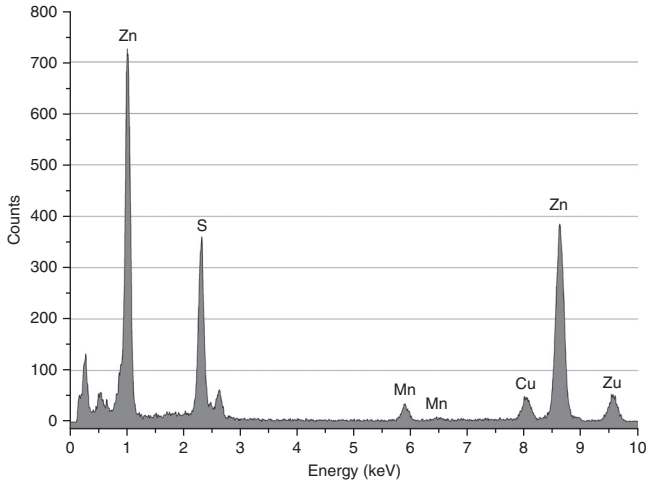

Figure 11 | EDX spectra for the ZnS:Mn QDs (Step 3B).

UV-visible spectra (**Fig. 12**) for the QDs before and after phase transfer show the characteristic bands for ZnS with a shoulder positioned at ~310 nm. The PL spectra (**Fig. 12**) for the QDs in $CHCl_3$ show a maximum intensity at 580 nm,

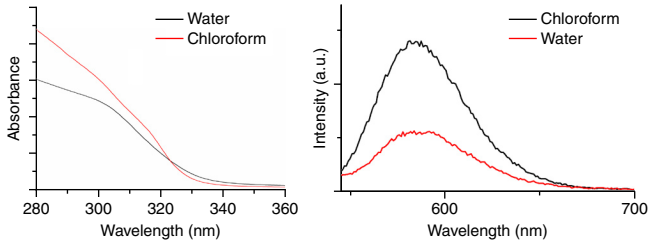

Figure 12 | Characterization of nanoparticles after phase transfer in Step 3B. The UV-visible spectra (left) and the PL spectra (right) before and after the phase-transfer process of the Mn-doped ZnS. The excitation wavelength for the PL spectra was 310 nm.

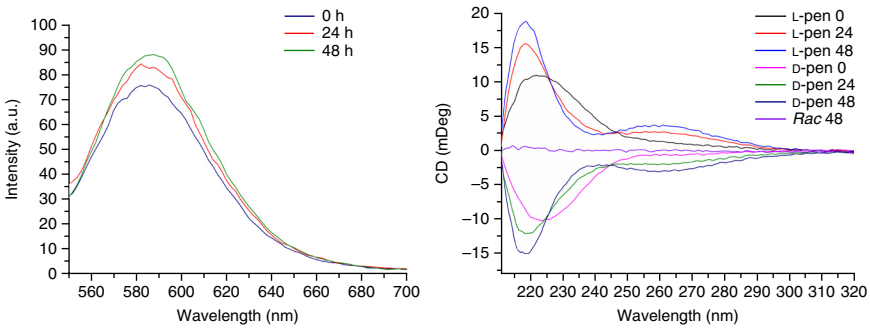

Figure 13 | Characterization of nanoparticles after 2 d of stirring, as described in Step 3B. Left, PL spectra of Mn:ZnS over 2 d of stirring (excitation wavelength 250 nm). Right, evolution of CD spectra of Mn:ZnS QDs during 2 d of stirring in the presence of an excess of ligands for D-penicillamine and L-penicillamine–stabilized QD solutions. a.u., arbitrary units.

which is characteristic of Mn doping in ZnS QDs. The intensity of the peak decreases by roughly 30% after the phase transfer to water. After the phase transfer, the QDs were stirred at 50 °C for 2 d; the PL spectra (**Fig. 13**) shows little to no loss of luminescence over the 2 d.

The CD spectra (**Fig. 13**) indicate a substantial change in CD responses over the 48-h heat treatment. The CD spectra initially (0 h) produce the characteristic bands of free penicillamine in solution. However, over time a new peak develops, starting ~300 nm, which is indicative of an interaction with the ZnS:Mn QDs. **Figure 14** clearly demonstrates a unique CD spectrum of the QDs after 48 h, which has bands in the band-edge region for ZnS.

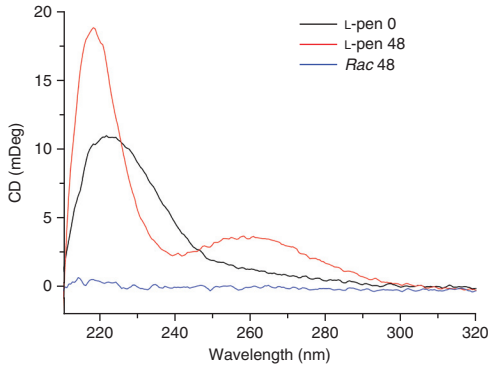

Figure 14 | CD spectra of ZnS:Mn over 2 d of stirring (Step 3B).

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AUTHOR CONTRIBUTIONS M.P.M., J.G. and A.L. performed the synthesis and characterization of quantum nanostructures. M.M. performed phase-transfer studies of chiral nanostructures. M.P.M., J.G., A.L. and Y.K.G. developed the protocols and wrote the manuscript.

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