Preparation of core-shell $NaGdF_4$ nanoparticles doped with luminescent lanthanide ions to be used as upconversion-based probes

Feng Wang[1,2], Renren Deng[3] & Xiaogang Liu[1,3,4]

[1]Institute of Materials Research and Engineering, Singapore, Singapore. [2]Department of Physics and Materials Science, City University of Hong Kong, Hong Kong SAR, China. [3]Department of Chemistry, National University of Singapore (NUS), Singapore, Singapore. [4]Center for Functional Materials, NUS (Suzhou) Research Institute, Suzhou, Jiangsu, China. Correspondence should be addressed to F.W. (fwang24@cityu.edu.hk) or X.L. (chmlx@nus.edu.sg).

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Sodium gadolinium fluoride ($NaGdF_4$) is an ideal host material for the incorporation of luminescent lanthanide ions because of its high photochemical stability, low vibrational energy and its ability to mediate energy exchanges between the lanthanide dopants. This protocol describes the detailed experimental procedure for synthesizing core-shell $NaGdF_4$ nanoparticles that incorporate lanthanide ions into different layers for efficiently converting a single-wavelength, near-IR excitation into a tunable visible emission. These nanoparticles can then be used as luminescent probes in biological samples, in 3D displays, in solar energy conversion and in photodynamic therapy. The $NaGdF_4$ nanoparticles are grown through co-precipitation in a binary solvent mixture of oleic acid and 1-octadecene. Doping by lanthanides with controlled compositions and concentrations can be achieved concomitantly with particle growth. The lanthanide-doped $NaGdF_4$ nanoparticles then serve as seed crystals for subsequent epitaxial growth of shell layers comprising different lanthanide dopants. The entire procedure for the preparation and isolation of the core-shell nanoparticles comprising two epitaxial shell layers requires ~15 h for completion.

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INTRODUCTION

Lanthanide-doped nanocrystals are an emerging class of nanomaterials that may be used in cells, tissues and living organisms as an alternative to organic dyes and semiconducting quantum dots as luminescent probes. These nanomaterials possess outstanding chemical and optical properties, including low cytotoxicity, large effective Stokes shifts, sharp emission bands, and high resistance to photobleaching and photochemical degradation[1,2]. More importantly, they can emit UV and visible light after excitation by near-IR (NIR) light, through a process known as photon upconversion[3]. These unique properties render lanthanide-doped nanocrystals particularly useful in 3D display, solar energy conversion and photodynamic therapy. As photon upconversion often involves excitation in a wavelength range in which absorption and scattering by biological samples are minimal, lanthanide-doped nanocrystals are also highly suitable as contrast agents for biological imaging in which background autofluorescence is not a concern[4–15]. Moreover, because of the outstanding magnetic properties of lanthanides, these lanthanide-doped nanocrystals offer the tantalizing possibility of MRI, bringing together luminescence and magnetism in a miniature dual-modality imaging system[16–18].

To fully realize the potential of lanthanide-doped nanocrystals for practical applications, one major challenge is to develop a general approach to tuning their color output after excitation by a single wavelength. In principle, lanthanide-doped nanoparticles are composed of an insulating host matrix and lanthanide dopants that are homogeneously distributed within the matrix. The optical emission from the nanocrystals is ascribed to the localized electronic transition within the *4f* orbitals of the lanthanide dopants. Owing to the weak coupling of the electronic transitions of the lanthanide dopants with the host lattice, variations in crystal shape and size have almost no effect on the luminescence profile of the nanocrystals, provided that the surface quenching effect is largely eliminated[19]. This behavior is in stark contrast with the size-tunable luminescence in semiconducting quantum dots as a result of dominant quantum-confinement effects[20–23].

Multicolor tuning of lanthanide-doped nanocrystals is typically achieved by modulating the relative intensities of the multipeak emissions through control of dopant composition and concentration[24–31]. For example, Tm^{3+}- and Er^{3+}-coactivated $NaYF_4$ nanocrystals are characterized by a dual-emission spectrum in which the emission intensity balance can be tuned by modifying the concentration ratios of Tm^{3+} and Er^{3+} ions[25]. The incorporation of Ce^{3+} at different concentrations into $NaYF_4$:Yb/Ho nanocrystals can also lead to a tunable optical emission owing to an energy exchange interaction between the Ce^{3+} and Ho^{3+} dopants[31]. However, different lanthanide dopant ions at high concentrations tend to quench upconverted luminescence by virtue of deleterious cross-relaxation processes. Therefore, the emission is typically tuned within a narrow spectral range because the selection of lanthanide dopants needs to meet stringent criteria in order to minimize luminescence quenching.

Recently, we have demonstrated that different sets of lanthanide ions can be doped into different layers of $NaGdF_4$-based core-shell nanoparticles with precise concentration, leading to exquisite control over dopant-dopant interaction and, consequently, unprecedented control over the characteristics of optical emissions[32,33]. Our design takes advantage of the core-shell approach that enables one to achieve spatial confinement of different lanthanide dopants in different core-shell layers, thus suppressing cross-relaxation and largely bypassing the stringent requirements for dopant composition and concentration. Importantly, the use of the $NaGdF_4$ host enables the transfer of the excitation energy to the activators (the lanthanide dopants) over a substantial length via energy migration through the gadolinium sublattice. Notably, the mentioned sublattice provides a bridge for

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the energy to be transferred between the lanthanides doped into different layers of the core-shell structure with minimal loss of the excitation energy. This effect can be harnessed to realize tunable upconversion emissions from a variety of lanthanide activators, including Eu^{3+}, Tb^{3+}, Dy^{3+} and Sm^{3+}, even at extremely low activator concentrations[32,33]. These phenomena are typically unexpected in conventional bulk materials comprising homogenously doped lanthanide activators.

The prerequisite for preparing $NaGdF_4$-based core-shell nanoparticles is to synthesize high-quality $NaGdF_4$ cores[34–40]. As a general rule of thumb, the precipitation of uniform nanoparticles from a mother solution is governed by a temporally discrete nucleation step followed by controlled growth of the nanoparticles[41–48]. Owing to the relatively low energy barrier between the nucleation and growth stages of $NaGdF_4$, the conventional synthesis of monodisperse $NaGdF_4$ nanoparticles typically requires strict attention to several experimental variables, such as solvent identity, reaction time, temperature and so on[49–51]. Here we present a facile and robust synthesis of lanthanide-doped $NaGdF_4$ nanoparticles through co-precipitation of lanthanide-oleate complex prompted by ammonium fluoride[19,32,33]. In this reaction, the nucleation and growth of the $NaGdF_4$ nanoparticles are separated by carrying out the two stages at low (50 °C) and elevated (280 °C) temperatures, respectively. The nucleation and growth rates can be further controlled by adjusting the concentration of the precipitator, leading to largely tunable particle sizes. Importantly, by changing the composition of the lanthanide-oleate precursor, different combinations of lanthanide ions can be easily incorporated into the nanoparticles.

The core-shell $NaGdF_4$ structure can be prepared by following the same procedure as the $NaGdF_4$ core, except for the inclusion of the presynthesized $NaGdF_4$ nanoparticles to the precursor solution as seed crystals before the addition of the precipitator. When compared with the conventional hot-injection method, the protocol described herein is much simpler, and it avoids the need for precise control of the feeding rate of the shell precursors[39,40]. In addition, the shell thickness can be tuned by adjusting the amount of shell precursors with respect to the core particles. By using the presynthesized core-shell $NaGdF_4$ nanoparticle as the seed crystal, the same epitaxial growth process can be repeated to fabricate a multilayered core-shell structure resembling that of an onion. Notably, we demonstrated that a $NaYF_4$ shell layer can also be grown on the $NaGdF_4$ nanoparticles through subtle adjustment of the shell growth condition[32]. The realization of the layer-by-layer hierarchical structure in nanoparticles without phase separation is essential for hosting multiple types of dopant ions with prespecified energy exchange interactions.

By tuning experimental variables such as the concentration ratios of oleic acid to 1-octadecene and fluoride to metal ions, the protocol for the synthesis of $NaGdF_4$ and its core-shell nanoparticles can be adapted to a wide variety of fluoride nanocrystals comprising rare earth, alkaline earth and alkali metal ions. Apart from the optical tunability offered by the dopant-host interactions, the use of different fluoride nanocrystals could enable the predetermination and control of the shape of the growing nanocrystals. Collectively, the nanocrystals with well-defined shapes and tunable optical emissions can assemble into ordered structures and even superlattices with fascinating properties that are desirable for advanced sensing and imaging applications. Compared with other optical nanomaterials, these fluoride nanocrystals are photochemically robust, and they function well over a wide range of conditions such as at high temperature and low pH.

Limitations

The protocol described herein is primarily intended to be used as a basis for doping lanthanide ions in fluoride-based core-shell nanocrystals. For nanocrystals involving oxide, chalcogenide or phosphate host lattices, the basic protocol design may require substantial modifications. On another matter, core-shell nanocrystals of relatively large size (>10 nm in diameter) are needed for maximal radiative transitions to achieve efficient emission. Even core-shell nanocrystals with the mentioned large sizes display, however, quite low upconversion luminescence efficiency (<2%) in most cases. To obtain an acceptable emission intensity level, the wavelength of the irradiating laser should match the narrow absorption band of Yb^{3+} at ~980 nm. However, the use of a 980-nm excitation source on a mixture of the nanocrystals dispersed in an aqueous solvent is likely to cause overheating-associated problems, because water molecules have a strong absorption peak at 980 nm (see ANTICIPATED RESULTS).

Experimental design

The $NaGdF_4$ nanoparticles are prepared by co-precipitation of the lanthanide fluorides with long-chain hydrocarbons (e.g., 1-octadecene) and unsaturated fatty acids, such as oleic acid. The unsaturated fatty acid is used as the surface ligand to control particle growth and subsequently stabilize the resultant nanoparticles against agglomeration. The core-shell structure is obtained via a two-step reaction through the use of the presynthesized core particle as the template for epitaxial growth of the shell layer (**Fig. 1**).

For the synthesis of monodisperse nanoparticles, an inorganic source of lanthanide acetate salts is first transferred into a flask containing 1-octadecene and oleic acid. The mixture is then heated to 150 °C, resulting in the formation of lanthanide-oleate coordination complexes. As the by-product of this reaction (i.e., acetic acid) has no noticeable effect on the subsequent particle growth, purification of the precursor solution is not necessary. The nucleation and growth of the $NaGdF_4$ nanocrystals are

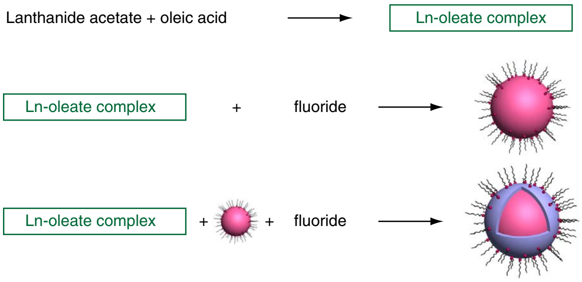

Figure 1 | Schematic illustration of the co-precipitation strategy for the synthesis of lanthanide-doped $NaGdF_4$ and its core-shell nanocrystals. Lanthanide-oleate precursors are prepared by the reaction of lanthanide acetate and oleic acid at 150 °C. The precipitation of the lanthanide-oleate precursors by fluoride at low temperature and subsequent annealing in high boiling solvent afford monodisperse $NaGdF_4$ nanocrystals.

triggered by the injection of a methanol solution of NaOH and NH_4F. The combined use of NaOH and NH_4F enables a finer control over the reaction rate than using NaF alone. It also minimizes corrosion of the glass flask.

The shell layer is grown by following a similar procedure to that just described, except that the presynthesized core particles are added to the solution before the injection of the NaOH/NH_4F solution. In this reaction, the core particles serve as foreign seeds that mediate epitaxial growth of the shell layer. As a result, the surface property of the seeding core particles can be harnessed as an extra parameter, in addition to common experimental conditions, to control the shape and size uniformity of the core-shell nanocrystals.

Most of the lanthanide reagents used in the synthesis are hygroscopic substances that readily absorb water from the surrounding atmosphere. Care must be taken in handling such compounds to avoid hydration of the lanthanide reagents, which leads to stoichiometry uncertainty. For certain types of nanoparticles with low dopant content, precise control over the dosage is required. To minimize the uncertainty incurred during weighing and transfer of the reagents, all chemicals are prepared in dilute stock solutions before use.

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MATERIALS

REAGENTS

- Oleic acid (technical grade, 90%; Sigma-Aldrich, cat. no. 364525)
  ▲ **CRITICAL** The quality of the oleic acid strongly affects the reactions. The same batch of reagents should be used in a set of closely related syntheses.
- 1-Octadecene (technical grade, 90%; Sigma-Aldrich, cat. no. O806)
- Methanol (reagent grade)
- Ethanol (reagent grade)
- Cyclohexane (reagent grade)
- Deionized (DI) water
- $Gd(CH_3CO_2)_3{\cdot}xH_2O$ (99.9% trace metals basis; Sigma-Aldrich, cat. no. 325678)
- $Yb(CH_3CO_2)_3{\cdot}4H_2O$ (99.9% trace metals basis; Sigma-Aldrich, cat. no. 326011)
- $Tm(CH_3CO_2)_3{\cdot}xH_2O$ (99.9% trace metals basis; Sigma-Aldrich, cat. no. 367702)
- $Tb(CH_3CO_2)_3{\cdot}xH_2O$ (99.9% trace metals basis; Sigma-Aldrich, cat. no. 325929)
- $Y(CH_3CO_2)_3{\cdot}xH_2O$ (99.9% trace metals basis; Sigma-Aldrich, cat. no. 326046)
- CH_3COOH (ACS reagent, ≥99.7%; Sigma-Aldrich, cat. no. 320099)
- NaOH (reagent grade, ≥98%; Sigma-Aldrich, cat. no. S5881)
- NH_4F (ACS reagent, ≥98%; Sigma-Aldrich, cat. no. 216011)
- (Optional) $GdCl_3{\cdot}xH_2O$ (99.99% trace metals basis), $YbCl_3{\cdot}6H_2O$ (99.9% trace metals basis), $TmCl_3{\cdot}6H_2O$ (99.99% trace metals basis), $TbCl_3{\cdot}6H_2O$ (99.9% trace metals basis), $YCl_3{\cdot}6H_2O$ (99.9% trace metals basis), $Nd(CH_3CO_2)_3{\cdot}xH_2O$ (99.9% trace metals basis) and $NdCl_3{\cdot}6H_2O$ (99.9% trace metals basis) (Sigma-Aldrich, cat. nos. 450855, 337927, 204668, 212903, 211648, 325805 and 289183, respectively)

EQUIPMENT

- Schlenk line with bubbler, cold trap and Dewar flask (e.g., Synthware, cat. nos. M250004, B257040, V165045 and F111900)
- Rotary vane pump (e.g., Edwards, RV3)
- Magnetic stirrer (e.g., Heidolph, MR Hei-Standard)
- Heating mantle with temperature controller (e.g., Misung, the E05 heating mantle and the TC200P temperature controller)
- Two-neck 50-ml round-bottom flask (e.g., Synthware, cat. no. F414950B)
- Flow control adapter (e.g., Synthware, cat. no. A702440B)
- Septum for fitting the temperature probe
- Teflon-coated, oval magnetic stir bar (dimensions: 20 × 10 mm)
- Three micropipettes (ranges: 20–200, 100–1,000 and 500–5,000 μl) with appropriate disposable tips
- Poly(propylene) centrifuge tubes (capacity 15 ml)
- Centrifuge (e.g., Hettich, EBA 20)
- Vortex mixer (e.g., Scientific Industries, Vortex-Genie 2)
- Ultrasonic cleaning bath (e.g., Elma, S30H)
- Laboratory balance (e.g., A&D, HR-200)
- (Optional) Crystallization dish (dimensions: 100 × 50 mm) and silicone fluid
- Transmission electron microscope (TEM, e.g., JEOL, JEM-1400) and carbon-coated copper TEM grids; for particle size and shape characterizations
- Photoluminescence spectrometer, quartz cuvette and 980-nm diode laser; for sample optical characterizations

REAGENT SETUP

Lanthanide stock solution Prepare the relevant stock solution of lanthanide complexes (0.2 M) by dissolving the particular lanthanide acetates in DI water with an ultrasonic cleaning bath. Note that the weights of the lanthanide acetates must be adjusted according to the certificates of analysis from the suppliers. The addition of an amount of acetic acid equal to 2% of the weight of lanthanide acetates facilitates the dissolution of the lanthanide acetates in DI water without affecting the properties of the nanocrystal products. Although highly water-soluble lanthanide chloride hydrates can also be used as starting materials, their use tends to lead to the formation of a large amount of NH_4Cl by-product, which requires a laborious purification process. The use of water-soluble lanthanide nitrite hydrates as starting materials should be avoided, as they may lead to degradation of organic solvents. Place the solutions in sealed containers and store them in a refrigerator at ~4 °C until needed. The lanthanide stock solution can be stored in these conditions for 12 months without producing any noticeable precipitation.

NaOH-methanol stock solution Prepare a 1 M solution of NaOH by dissolving the appropriate amount of NaOH in methanol with an ultrasonic cleaning bath. Place the solution in a sealed plastic container and store it in a refrigerator at ~4 °C until needed. The NaOH methanol stock solution can be stored in these conditions for 1 month without producing any noticeable precipitation.

NH_4F-methanol stock solution Prepare a 0.4 M solution of NH_4F by dissolving the appropriate amount of NH_4F in methanol with an ultrasonic

Figure 2 | Photograph of the experimental setup for the preparation of the $NaGdF_4$ nanocrystals. Please note that the vacuum/inert gas adapter is not connected to the flask until the reaction mixture is to undergo degassing (see Steps 6, 19 and 32); this process facilitates the removal of the water and methanol added to the reaction mixture as solvents of the reagents. The setup should be placed in a fume hood.

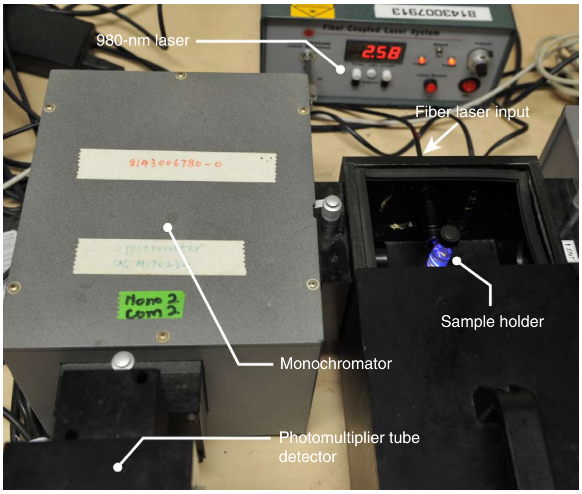

Figure 3 | Photograph of the experimental setup for the measurement of upconversion emission spectra. Note that the 980-nm continuous-wave laser is directed into the sample holder through an optical fiber.

cleaning bath. Place the solution in a sealed plastic container and store it in a refrigerator at ~4 °C until needed. The NH_4F methanol stock solution can be stored in these conditions for 1 month without producing any noticeable precipitation.

EQUIPMENT SETUP

Nanocrystal synthesis setup This setup consists of a two-neck, 50-ml round-bottom flask equipped with a vacuum/inert gas adapter, a septum, a stir bar and a temperature probe. To avoid cross-contamination between the metallic temperature probe and the reaction solution, cap the temperature probe with a customized glass tube before placing it in the reaction solution. A magnetic stirrer is used to provide the stirring action in the flask. A heating mantle, equipped with a temperature controller, is used to apply heat to the flask. A typical setup is shown in **Figure 2**. Notably, the reaction flask can be heated in an oil bath for the preparation of lanthanide-oleate complexes (Steps 2 and 3, 14 and 15, and 27 and 28), which requires a relatively low reaction temperature. When an oil bath is used, the magnetic stirrer should also function as a heating device.

TEM setup The TEM measurements are carried out on a JEM-1400 TEM instrument (JEOL). The microscope is operated under an accelerating voltage of 120 kV. Samples are prepared for imaging by slowly drying nanoparticles on a copper grid coated with a thin layer of carbon. To avoid particle agglomeration, a dilute solution of nanoparticles in cyclohexane can be used.

Upconversion luminescence spectrometer setup A modified photoluminescence spectrometer coupled with a 980-nm continuous-wave laser is used for the luminescence measurements. A typical setup is shown in **Figure 3**. In this setup, a fiber-coupled 980-nm laser (CNI, FC-980-3W) is used as the excitation source for the upconversion excitation. The laser is guided into a homemade sample holder through a laser adapter along with a beam collimator. A DM150i monochromator equipped with a R928 photon counting photomultiplier is used to record the upconversion emission spectra. To avoid the entrance of the excitation laser into the detector, the excitation and the detection chambers are operated using a 90° measurement geometry. We recommend placing a 900-nm short-pass filter between the sample and the monochromator to prevent the scattered laser beam from entering the detection system. **! CAUTION** Laser beam can seriously damage the eyes and skin. Wear protective glasses or goggles to prevent direct exposure to the beam.

PROCEDURE

Synthesis of $NaGdF_4$:Yb/Tm nanocrystals ● TIMING 5 h

1| At room temperature (23–25 °C), pipette 1 ml of the $Gd(CH_3CO_2)_3$ stock solution, 0.98 ml of the $Yb(CH_3CO_2)_3$ stock solution, 0.02 ml of the $Tm(CH_3CO_2)_3$ stock solution, 4 ml of oleic acid and 6 ml of 1-octadecene into a 50-ml flask (see 'Nanocrystal synthesis setup' in Equipment Setup).

2| Fit the flask with a thermocouple temperature sensor and heat the solution to 150 °C, and keep it at that temperature for 40 min while stirring.
! CAUTION Set a moderate stirring speed to avoid splashing of the solution. Note that the flask is left uncapped at this point to remove residual moisture. The splashed solution may cause severe burns upon eye or skin contact. Use caution and wear appropriate PPE.

3| Remove the heating mantle and allow the reaction mixture to cool down slowly to room temperature while stirring.
? TROUBLESHOOTING
■ PAUSE POINT The flask can be kept at room temperature for several hours without decomposition of lanthanide-oleate precursors.

4| At room temperature, pipette 1 ml of NaOH-methanol stock solution and 3.3 ml of NH_4F-methanol stock solution into a 15-ml centrifuge tube. Seal the tube properly and mix the solution by vortexing for 10 s. Then quickly inject the mixture into the reaction flask.
▲ CRITICAL STEP The mixing of NaOH and NH_4F in methanol solution will result in precipitation of NaF. To prevent solid NaF from sticking to the wall of the centrifuge tube, the solution must be injected into the flask immediately after mixing. The amount of precipitators added in this step is crucial to the formation of $NaGdF_4$ nanocrystals with high size and shape uniformity. For instance, a decrease in the amount of NH_4F stock solution from 3.3 to 2.7 ml can result in an increase in the nanocrystal size from 15 nm to 30 nm (see ANTICIPATED RESULTS).

5| Increase the solution temperature to 50 °C and keep the reaction at this temperature for 30 min.
▲ CRITICAL STEP Do not set the temperature above 50 °C, as doing so will promote crystal growth (as opposed to simple nucleation). Keep the reaction at 50 °C for no less than 30 min to ensure completion of nucleation. We suggest calibrating the thermocouple with a mercury thermometer before implementing the instructions provided in this step.

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6| Increase the temperature to 100 °C. Next, connect the flask to a Schlenk line with a dual vacuum/gas manifold and keep the reaction mixture under vacuum (0.01 mbar) for 10 min.
▲ **CRITICAL STEP** Open the stopcock that controls access to the gas and vacuum lines slowly when degassing under vacuum, as a sudden decrease in pressure will result in the reaction mixture flowing into the vacuum line.

7| Switch the position of the stopcock so as to fill the flask with argon, and then raise the temperature to 280 °C at a heating rate of 10 °C/min. Keep the reaction mixture at 280 °C for 1.5 h.
▲ **CRITICAL STEP** Switch the two-way stopcock slowly when connecting the flask to the argon gas line. Otherwise, the bubbler oil will be sucked into the Schlenk line. Increasing the flow rate of the argon gas can help prevent this from occurring to some extent.

8| Remove the heating mantle and let the reaction mixture cool down slowly to room temperature while stirring.
? TROUBLESHOOTING
■ **PAUSE POINT** The flask can be kept at room temperature for several hours without affecting the quality of $NaGdF_4$ nanocrystals.

9| Transfer the contents of the flask into a 15-ml centrifuge tube. Rinse the flask with 5 ml of ethanol and transfer the solution to the centrifuge tube. Spin down the product at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature, and then discard the supernatant.
? TROUBLESHOOTING

10| Add 4 ml of cyclohexane to the centrifuge tube and agitate it with a vortex mixer to re-disperse the product. Subsequently, add 8 ml of ethanol to the tube and vortex the mixture thoroughly. Spin down the product at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature, and then discard the supernatant.
? TROUBLESHOOTING

11| Repeat Step 10, replacing the 8 ml of ethanol with 4 ml of ethanol and 4 ml of methanol.

12| Disperse the $NaGdF_4$ nanocrystals present in the centrifuge tube in 4 ml of fresh cyclohexane. The as-synthesized core nanocrystals are spherical with an average diameter of ~15 nm (see ANTICIPATED RESULTS). The preparation of monodisperse core particles is the prerequisite for synthesizing uniform core-shell particles. We strongly recommend that researchers control the quality of the core particles with respect to their uniform size and spherical shape before proceeding to the next step.
? TROUBLESHOOTING
■ **PAUSE POINT** A colloid of $NaGdF_4$ nanocrystals in cyclohexane can be temporarily (~24 h) stored in a sealed poly(propylene) centrifuge tube. For prolonged storage of the nanocrystal solution, we recommend using glass vessels, as cyclohexane can cause polymeric containers to swell. A cyclohexane colloid of $NaGdF_4$ nanocrystals in a properly sealed glass vial can be stored in the refrigerator (4 °C) for up to a few months, without marked changes to their size, morphology and optical properties. Note that colloidal nanocrystals tend to agglomerate after prolonged storage. Individual nanocrystals can be easily recovered from the agglomeration through sonication.

Synthesis of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb core-shell nanocrystals ● TIMING 5 h

13| At room temperature, pipette 1.7 ml of $Gd(CH_3CO_2)_3$ stock solution, 0.3 ml of $Tb(CH_3CO_2)_3$ stock solutions, 4 ml of oleic acid and 6 ml of 1-octadecene into a 50-ml flask.

14| Fit the flask with a thermocouple temperature sensor, and then heat the reaction mixture to 150 °C and keep it at that temperature for 40 min under stirring.
! CAUTION Set a moderate stirring speed to avoid splashing of the solution. Note that the flask is left uncapped at this point to remove residual moisture. The splashed solution may cause severe burns on eye or skin contact. Use caution and wear appropriate PPE.

15| Remove the heating mantle and allow the reaction mixture to cool down slowly to room temperature while stirring.
? TROUBLESHOOTING
■ **PAUSE POINT** The flask can be kept at room temperature under stirring for several hours without affecting the quality of lanthanide-oleate precursors.

16| At room temperature, inject the core particles obtained in Step 12 into the reaction flask.

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17| At room temperature, pipette 1 ml of NaOH methanol stock solution and 3.3 ml of NH_4F methanol stock solution into a 15-ml centrifuge tube. Seal the tube properly and mix the solution by vortexing for 10 s. Then quickly inject the mixture into the reaction flask from Step 16.
▲ **CRITICAL STEP** Mixing of NaOH and NH_4F in methanol will result in precipitation of NaF. To prevent solid NaF from sticking to the wall of the tube, inject the solution into the flask immediately after mixing. In contrast to the synthesis of core nanocrystals, the amount of precipitator added in this step mainly affects the size (or shell thickness) of the resulting core-shell $NaGdF_4$ nanocrystals, not their shape (see ANTICIPATED RESULTS). The marginal shape-dependence on the precipitator content in the synthesis of core-shell nanocrystals is probably because of the fact that a crystal growth dynamic that is different from that which controls nucleation dominates in the epitaxial growth process.

18| Increase the reaction mixture temperature to 50 °C and keep it at this temperature for 30 min.

19| Increase the temperature to 100 °C. Then connect the flask to the Schlenk line and place the reaction mixture under vacuum (0.01 mbar) for 10 min.
▲ **CRITICAL STEP** Open the stopcock that controls access to the gas and vacuum lines slowly when degassing under vacuum, as a sudden decrease in pressure will result in the reaction mixture flowing into the vacuum line.

20| Switch the position of the stopcock so as to fill the flask with argon, and raise the temperature to 280 °C at a heating rate of 10 °C/min. Keep the reaction mixture at this temperature for 1.5 h.
▲ **CRITICAL STEP** Switch the two-way stopcock slowly when connecting the flask to the argon gas line. Otherwise, the bubbler oil will be sucked into the Schlenk line. Increasing the flow rate of the argon gas can help prevent this from occurring to some extent.

21| Remove the heating mantle and let the reaction mixture cool down slowly to room temperature while stirring.
? TROUBLESHOOTING
■ **PAUSE POINT** The flask can be kept at room temperature under stirring for several hours without affecting the quality of core-shell $NaGdF_4$ nanocrystals.

22| Transfer the content of the flask into a 15-ml centrifuge tube. Rinse the flask with 5 ml of ethanol and transfer the washings into the centrifuge tube. Spin down the centrifuge tube at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature and discard the supernatant.

23| Add 4 ml of cyclohexane to the centrifuge tube, and agitate it with a vortex mixer to re-disperse the product. Subsequently, add 8 ml of ethanol to the tube and agitate the reaction mixture via vortexing. Spin down the product at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature, and then discard the supernatant.
? TROUBLESHOOTING

24| Repeat Step 23, replacing the 8 ml of ethanol with 4 ml of ethanol and 4 ml of methanol.

25| Disperse the core-shell $NaGdF_4$ nanocrystals in 4 ml of fresh cyclohexane and store the resulting colloid in a well-sealed vial. The core-shell nanocrystals essentially preserve the shape of the core nanocrystals with a mono-modal size distribution. The particle size (or shell thickness) of the core-shell nanocrystals can be tuned by varying the mass ratio of the shell to the core (see ANTICIPATED RESULTS).
? TROUBLESHOOTING
■ **PAUSE POINT** A colloid of core-shell $NaGdF_4$ nanocrystals in cyclohexane can be stored in the refrigerator (4 °C) for up to a few months without particle agglomeration or loss of the luminescent properties. The nanocrystals can also be extracted from the colloid via centrifugation (Step 22), and they can be stored in solid form at room temperature for up to 2 years without a noticeable change in their optical properties. The nanocrystals later can be re-dispersed in nonpolar solvents such as cyclohexane by agitating with an ultrasonic cleaning bath.

Synthesis of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb@$NaYF_4$ core-shell-shell nanocrystals ● TIMING 5 h

26| At room temperature, pipette 2 ml of $Y(CH_3CO_2)_3$ stock solution, 3 ml of oleic acid and 7 ml of 1-octadecene into a 50-ml round-bottom flask.
▲ **CRITICAL STEP** The volume ratio of oleic acid to 1-octadecene should be slightly lower than that for the growth of $NaGdF_4$ shells. We have observed that a high concentration of oleic acid prevents the formation of uniform $NaYF_4$ shells, probably owing to dissimilar chemical reactivity of yttrium and gadolinium in the mentioned conditions.

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27| Fit the flask with a thermocouple, heat the flask to 150 °C and keep it at that temperature for 40 min while stirring.
! CAUTION Set a moderate stirring speed to avoid splashing of the solution. Note that the flask is left uncapped at this point to remove residual moisture. The splashed solution may cause severe burns following eye or skin contact. Use caution and wear appropriate PPE.

28| Remove the heating mantle and allow the reaction mixture to cool down slowly to room temperature while stirring.
? TROUBLESHOOTING
■ **PAUSE POINT** The flask can be kept at room temperature under stirring for several hours without affecting the quality of lanthanide-oleate precursors.

29| At room temperature, inject the core-shell particles obtained in Step 25 into the reaction flask.

30| At room temperature, pipette 1 ml of NaOH methanol stock solution and 3.3 ml of NH_4F methanol stock solution into a 15-ml centrifuge tube. Seal the tube properly and mix the solution via vortexing for 10 s. Next, quickly inject the mixture into the reaction flask.
▲ **CRITICAL STEP** Mixing NaOH and NH_4F in methanol solution will result in precipitation of NaF. To prevent the NaF residue from building up on the wall of the tube over time, inject the solution into the flask immediately after mixing. Similarly to the synthesis of core-shell nanocrystals, the amount of precipitator added in this step will affect the layer thickness of the second shell of the resulting core-shell-shell nanocrystals (see ANTICIPATED RESULTS).

31| Increase the temperature of the reaction mixture to 50 °C and keep it at this temperature for 30 min.

32| Increase the temperature to 100 °C, and then connect the flask to a Schlenk line and place the reaction mixture under vacuum (0.01 mbar) for 10 min.
▲ **CRITICAL STEP** Open the stopcock slowly when degassing under vacuum, as a sudden decrease in pressure will result in the reaction mixture flowing into the vacuum line.

33| Switch the stopcock so as to fill the flask with argon, and then raise the temperature to 280 °C at a heating rate of 10 °C/min. Keep the reaction mixture at this temperature for 1 h.
▲ **CRITICAL STEP** Switch the two-way stopcock slowly when connecting to the argon gas; otherwise, the bubbler oil will be sucked into the Schlenk line. Increasing the flow rate of the argon gas can help prevent this from occurring to some extent.

34| Remove the heating mantle and let the reaction mixture cool down slowly to room temperature under stirring.
? TROUBLESHOOTING
■ **PAUSE POINT** The flask can be kept at room temperature under stirring for several hours without affecting the quality of the core-shell $NaGdF_4$ nanocrystals.

35| Transfer the contents of the flask into a 15-ml centrifuge tube. Rinse the flask with 5 ml of ethanol and transfer the washings into the centrifuge tube. Spin down the tube at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature and discard the supernatant.

36| Add 4 ml of cyclohexane to the centrifuge tube, and agitate it with a vortex mixer to re-disperse the product. Subsequently, add 8 ml of ethanol to the tube and agitate the solution again via vortexing. Spin down the tube at 6,000 r.p.m. (~3,500*g*) for 3 min at room temperature and discard the supernatant.
? TROUBLESHOOTING

37| Repeat Step 36, replacing the 8 ml of ethanol with 4 ml of ethanol and 4 ml of methanol.

38| Disperse the core-shell-shell nanocrystals in 4 ml of fresh cyclohexane, and then store the colloid in a well-sealed vial. The core-shell-shell nanocrystals essentially preserve the shape of the core nanocrystals with a narrow size distribution.
? TROUBLESHOOTING
■ **PAUSE POINT** A colloid of $NaGdF_4$@$NaGdF_4$@$NaYF_4$ core-shell-shell nanocrystals in cyclohexane can be stored in a refrigerator (4 °C) for up to a few months without any notable sign of particle aggregation or degradation. The nanocrystals can also be stored in solid form for up to 2 years at room temperature by extracting the nanocrystals from the colloid via centrifugation (Step 35). The re-dispersion of the nanocrystals can be readily achieved by ultrasonic agitation in nonpolar solvents such as cyclohexane.

TEM characterization of the as-prepared nanocrystals ● TIMING 1 h

39| Add 90 µl of cyclohexane to 10-µl aliquots of each of the cyclohexane colloids containing the $NaGdF_4$ nanocrystals obtained in Steps 12, 25 and 38. Next, deposit the diluted nanocrystal colloid onto a copper TEM grid coated with carbon.

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40| Air-dry the nanocrystals deposited on the copper grid at room temperature for 15 min.
■ **PAUSE POINT** The nanocrystals can be stored at room temperature for several months without any noticeable changes in morphology.

41| Image the nanocrystals by TEM, using a JEM-1400 instrument.

Upconversion luminescence measurements of the as-prepared nanocrystals ● TIMING 30 min
42| Clean and dry a quartz cuvette. Please note that a glass cuvette may also be used to hold samples for measuring the luminescence spectra for wavelengths above 350 nm.

43| Fill the cuvette with a cyclohexane colloid of the samples obtained in Steps 12, 25 or 38.

44| Place the cuvette in the sample holder of the spectrometer (**Fig. 3**). Measure the sample emission under 980-nm laser excitation.
! CAUTION The laser beam can seriously damage the eyes and skin. Wear protective goggles and avoid direct exposure to laser light.
▲ **CRITICAL STEP** The emission spectra are strongly affected by the laser power. We excited the nanocrystals at a power density of 15 W/cm^2. We suggest a minimum excitation power density of 10 W/cm^2 for the ANTICIPATED RESULTS below to be observed.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reason	Solution
3, 15, 28	Cloudy solution	Presence of oxidized contaminants or impurities in low-grade reagents	Use high-grade lanthanide salt precursors Heat the solution at high temperature under an inert atmosphere
	Noticeable solid residues	Degraded oleic acid	Use fresh oleic acid. Note that oleic acid should be properly sealed and stored in the refrigerator (4 °C) until needed to prevent rancidification. Degraded oleic acid may also affect the size of the nanocrystals
8, 21, 34	Black-colored products form	There might be a leak in the vacuum line or caused by insufficient degassing in Steps 5 or 19	Pay careful attention to the digital monitor to ensure that there are no leaks in the Schlenk line. Repeat the degassing process several times to ensure that oxygen and moisture are sufficiently excluded in the reaction flask
9	Phase separation after the addition of ethanol	Inadequate amounts of oleic acid in the reaction medium	Make sure that the solvent comprises 40% in volume of oleic acid and 60% in volume of 1-octadecene
	No solid precipitates	Low reaction temperature	Increase the temperature to at least 230 °C to promote the growth of nanocrystals
10, 23, 36	No nanoparticle precipitate forms at the bottom of the centrifuge tube after centrifugation	High water content in the ethanol	Use high-purity (>98 %) ethanol for nanocrystal purification
12	High polydispersity in the particle size	Incorrect amounts of NH_4F are added in Step 4	Keep the volume of NH_4F methanol solution between 2.7 and 3.3 ml. Adding substantially lower or higher volumes of the NH_4F stock solution will lead to nucleation and crystal growth processes that are difficult to control
	The nanocrystals tend to precipitate in non-polar solvent such as cyclohexane	Improper amount of NaOH is added in Step 4	Use 1 ml of the NaOH-methanol stock solution. A decrease in NaOH content will result in a sharp increase in the nanoparticle size
25, 38	Bimodal size distribution of the nanocrystals	Insufficient amounts of core or core-shell particles are added for seeded crystal growth in Steps 16 or 29	Increase the concentration of the core particles to suppress the phase separation Prolong the reaction in Steps 20 or 33 to promote the formation of uniform core-shell nanocrystals through Ostwald ripening

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● TIMING

Steps 1–12, synthesis of $NaGdF_4$:Yb/Tm nanocrystals: 5 h

Steps 13–25, synthesis of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb core-shell nanocrystals: 5 h

Steps 26–38, synthesis of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb@$NaYF_4$ core-shell-shell nanocrystals: 5 h

Steps 39–41, TEM characterization of the as-prepared nanocrystals: 1 h

Steps 42–44, upconversion luminescence measurements of the as-prepared nanocrystals: 30 min

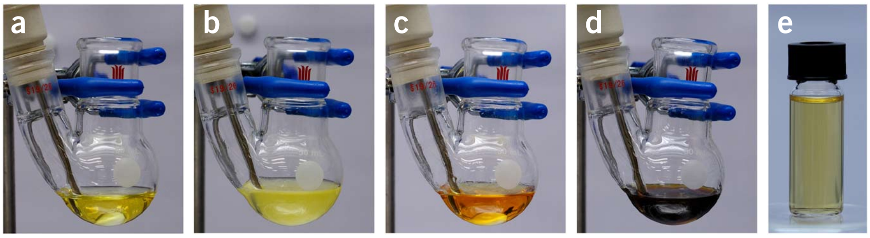

Figure 4 | Photographs of the reaction mixture at various stages of the preparation. (**a**) The lanthanide oleate precursors in oleic acid and 1-octadecene, corresponding to the solution obtained in Step 3. (**b**) The precursor solution upon addition of NaOH and NH_4F (Step 4). (**c**) After heating at 50 °C for 40 min (Step 5). (**d**) After heating at 280 °C for 1.5 h (Step 8). (**e**) A colloid of re-dispersed nanoparticles in cyclohexane (2% (wt/vol)) after purification (Step 12).

ANTICIPATED RESULTS

After heating the lanthanide aqueous solution in a mixture of oleic acid and 1-octadecene (Step 3), a clear yellow solution should be obtained (**Fig. 4a**). Upon injection of the methanol solution of NaOH and NH_4F (Step 4), the solution containing the lanthanide salt is expected to change in appearance from transparent to opaque (**Fig. 4b**). As the nucleation reaction proceeds at 50 °C, the reaction mixture regains its transparency; this process is accompanied by a darkening in color (**Fig. 4c**). After heating at 280 °C (Step 8), the reaction mixture should appear dark brown, and it should contain crude $NaGdF_4$ nanocrystals (**Fig. 4d**). The cyclohexane dispersion of the nanocrystals after washing (Step 12) should display a light yellow color under ambient light (**Fig. 4e**). The nanocrystals are spherical in shape with a diameter of ~15 nm (**Fig. 5**). The core nanocrystals emit blue upconversion luminescence upon excitation at 980 nm with a diode laser (**Fig. 5b**), resulting from a sequential energy transfer process from Yb^{3+} to Tm^{3+}. The particle size can be tuned by adjusting the volume of the NH_4F solution added in Step 4 (**Fig. 6a**–**c**).

Similar observations are expected in Steps 13–25 and Steps 26–38 as in the synthesis of core-shell and core-shell-shell nanoparticles. One notable difference is that the color of the precursor solution comprising the Tb-oleate complex is darker than that for preparing the core nanocrystals. The core-shell and core-shell-shell nanocrystals essentially preserve the morphology of the core particles (**Fig. 5c**,**e**). Upon excitation at 980 nm with a diode laser, the core-shell nanocrystals emit a green color with much higher intensity than the core particles (**Fig. 5d**). The enhanced optical emission is attributed to suppressed surface quenching to Yb^{3+} and Tm^{3+} ions that are embedded in the interior of the core-shell nanocrystals. The color change is due to Gd sublattice–mediated energy transfer from Tm^{3+} to Tb^{3+}, resulting in green emission of the latter. The inert $NaYF_4$ shell layer on the $NaGdF_4$@$NaGdF_4$ core-shell nanocrystals prevents the surface quenching of the excitation energy stored in the Gd sublattice, further enhancing the intensity of the green light emitted by Tb^{3+} (**Fig. 5f**).

The particle size (or shell thickness) of the core-shell nanocrystals can be principally controlled by adjusting the volume of the core particle colloid added in Step 16 (**Fig. 6d**,**e**). High-quality shells with tunable compositions can also be readily prepared by varying lanthanide elements in the shell precursors (**Fig. 6f**,**g**). Although we detail the protocol for preparing core-shell $NaGdF_4$ nanoparticles, this protocol can be implemented to prepare other lanthanide-doped core-shell systems. However, because the nucleation and crystal growth processes strongly depend on particle compositions, experimental variables such as the ratios of oleic acid/1-octadecene and fluoride/metal ions should be carefully adjusted in order to form other types of nanoparticles (**Table 2**). It is also worth mentioning that anisotropic shell growth may become prominent for certain types of core particles because of the marked difference in surface energy of different crystal facets. As the core-shell structure is mainly

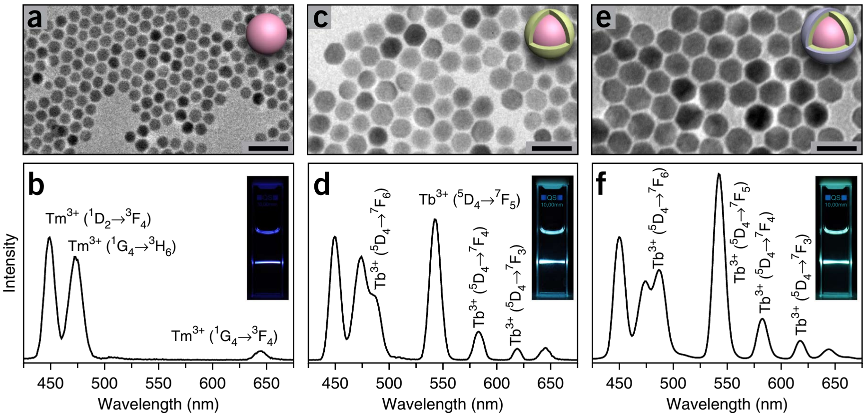

Figure 5 | Characterization of the $NaGdF_4$ nanocrystals. (**a**,**b**) TEM image and emission spectrum of the $NaGdF_4$:Yb/Tm core nanocrystals. (**c**,**d**) TEM image and emission spectrum of the $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb core-shell nanocrystals. (**e**,**f**) TEM image and emission spectrum of the $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb@$NaYF_4$ core-shell-shell nanocrystals. Insets in **b**,**d**,**f** show luminescence photographs of the relevant nanocrystal colloids. Scale bars, 50 nm.

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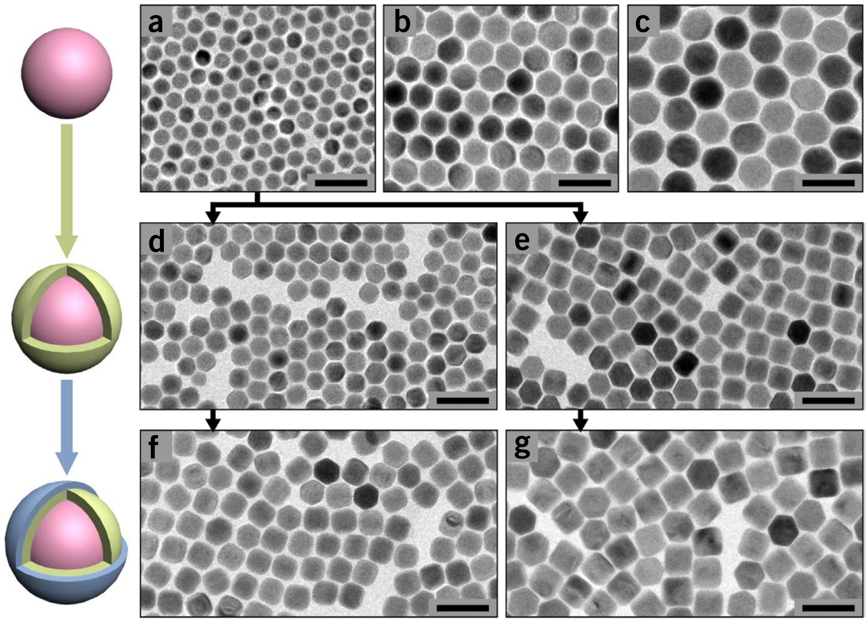
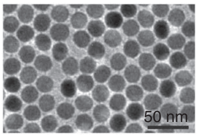
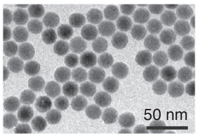
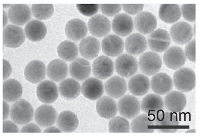
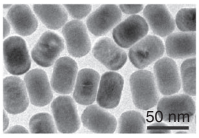


Figure 6 | Size-tuning of the $NaGdF_4$ nanocrystals. (**a**–**c**) TEM images of $NaGdF_4$:Yb/Tm nanocrystals prepared by adding 3.3, 3.1 and 2.7 ml of NH_4F/methanol solutions in Step 4, respectively. (**d**,**e**) TEM images of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb core-shell nanocrystals prepared by adding 50 and 25 mg of 15-nm core particles in Step 16, respectively. (**f**,**g**) TEM images of $NaGdF_4$:Yb/Tm@$NaGdF_4$:Tb@$NaGdF_4$ core-shell-shell nanocrystals prepared by following the inner shell coating process (Steps 13–25) through the use of the core-shell nanocrystals as the seeds and $Gd(CH_3CO_2)_3$ stock solution as the shell precursor. Scale bars, 50 nm.

used to spatially partition the lanthanide dopant ions, the anisotropic shell coating is equally useful for modulating optical emission. For example, by using an anisotropic core-shell-shell nanostructure comprising a $NaYbF_4$:Nd core and successively coated $NaGdF_4$:Yb/Tm and $NaGdF_4$:Tb shells, tunable upconversion emission can be realized with biocompatible 800-nm excitation, which avoids the overheating effect typically associated with using a 980-nm wavelength to achieve excitation of conventional upconversion nanoparticles[52].

TABLE 2 | Typical experimental settings for preparing nanoparticles of varying compositions.

$NaGdF_4$:Yb/Tm/Y (49/1/20%)[a]	$NaYF_4$:Yb/Tm (29/1%)[a]	$NaYF_4$:Yb/Tm (49/1%)[a]	$NaYF_4$:Yb/Tm (49/1%)@$NaYF_4$[a]
OA/ODE = 4/6	OA/ODE = 3/7	OA/ODE = 3/7	OA/ODE = 3/7
F/RE = 3.9	F/RE = 4	F/RE = 4	F/RE = 4
Average diameter = 22 nm	Average diameter = 18 nm	Average diameter = 26 nm	Average size = 43 × 26 nm
			

OA, oleic acid; ODE, 1-octadecene; F, fluoride; RE, rare-earth ions.

[a] The percentages refer to the mole proportions of the dopant ions among all rare earth elements in the host nanocrystals.

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