ARTICLE

A Four-Color Colloidal Multiplexing Nanoparticle System

Oliver Ehlert,[†] Ralf Thomann,[‡] Masih Darbandi,[†] and Thomas Nann[§,*]

[†]Freiburg Materials Research Center, University of Freiburg, Germany, [‡]Department of Macromolecular Chemistry, University of Freiburg, Germany, and [§]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, United Kingdom

ABSTRACT Any labeling with multicolor markers can be affected by the autofluorescence of biological tissue due to the UV or blue light excitation sources, or the results are affected by fluorescence resonance energy transfer. In this work, we present novel IR-to-vis upconverting nanoparticles of different rare earth metal dopants. With a single excitation source of 980 nm, four different colors of nanocrystals can be spectrally separated under multiplexing conditions. The particles were phase transferred into polar solvents by means of silica encapsulation and were characterized by transmission electron microscopy, X-ray diffraction, and photoluminescence spectroscopy.

KEYWORDS: upconversion · colloidal nanoparticles · multicolor multiplexing · silica encapsulation

Nanocrystalline materials are widely used for biological imaging. The advantages of semiconductor nanocrystals (NCs) such as CdSe/ZnS, CdSe/ZnS/SiO_2[1–3] or InP/ZnS[4] lie in their stability against photobleaching, size-dependent emission wavelengths, high brilliance, wide excitation range, and small emission line bandwidth.[5] Recently, we reported on the synthesis of Mn-doped ZnS nanocrystals[6] for biological applications and Cu or Pb (co)doped ZnS NCs[7] as less-toxic alternatives for the cadmium-based counterparts. Because these particles have to be excited with blue or UV light, biological tissue shows its typical autofluorescence if investigated by means of fluorescence microscopy. A colored nanoparticle system that is excited at wavelengths at which biological tissue does not show autofluorescence would provide a powerful tool in the hands of researchers to increase the signal-to-noise ratio in imaging or fluorescence detection of biological samples. The excitation wavelength should be as "red" as possible (or infrared) to prevent tissue or synthetic material from being excited. Furthermore, infrared light penetrate more deeply into biological tissue, thus enabling noninvasive bioimaging.

Such a system could be based upon nanoparticles that show near-infrared-to-visible (NIR-to-vis) upconversion luminescence. Consult Auzel[10] for a review on different upconversion systems. Some of these particles in the $NaYF_4$:Yb system were reported by Haase and co-workers by precipitation methods in aqueous solutions.[8,9] However, these particles possess the cubic structure, which is known to be less effective during the upconversion processes. Recently, Mai *et al.* reported the synthesis of hexagonal and cubic phased $NaREF_4$ (RE = Y, Pr to Lu) with polyhedra-, rod-, plate-, and dot-like morphology, as well as $NaYF_4$:Yb nanoparticles codoped with Er or Tm with polyhedra- and plate-like morphology.[11] This synthesis method is based on the thermal decomposition of metal trifluoroacetates (TFAs) in long-chain capping agents. Because codoping in the $NaYF_4$:Yb system would mean that four different metal TFAs have to be handled, we exploited Mai *et al.*'s procedure for the synthesis and doping of hexagonal-phased $NaYbF_4$ nanoparticles. In this system, only three components have to be handled during the synthesis and, therefore, the doping procedure. Here, the Yb^{3+} ions, which take part as absorbers in the upconversion processes at 980 nm, form the host lattice. We present nanoparticles in the size range of about 20 nm from blue- and IR-emitting $NaYbF_4$:Tm^{3+}, green (and slightly red)-emitting $NaYbF_4$:Ho^{3+}, green- and red-emitting $NaYbF_4$:Er^{3+}, and blue-, green-, red-, and IR-emitting $NaYF_4$:Yb^{3+}. Additionally, $NaYF_4$:Yb, Tb and $NaYbF_4$:Tb, NCs with green and blueish upconverted photoluminescence (UC-PL) at room temperature, respectively, are pre-

*Adress correspondence to T.Nann@uea.ac.uk.

Received for review September 21, 2007 and accepted November 29, 2007.

10.1021/nn7002458 CCC: $40.75

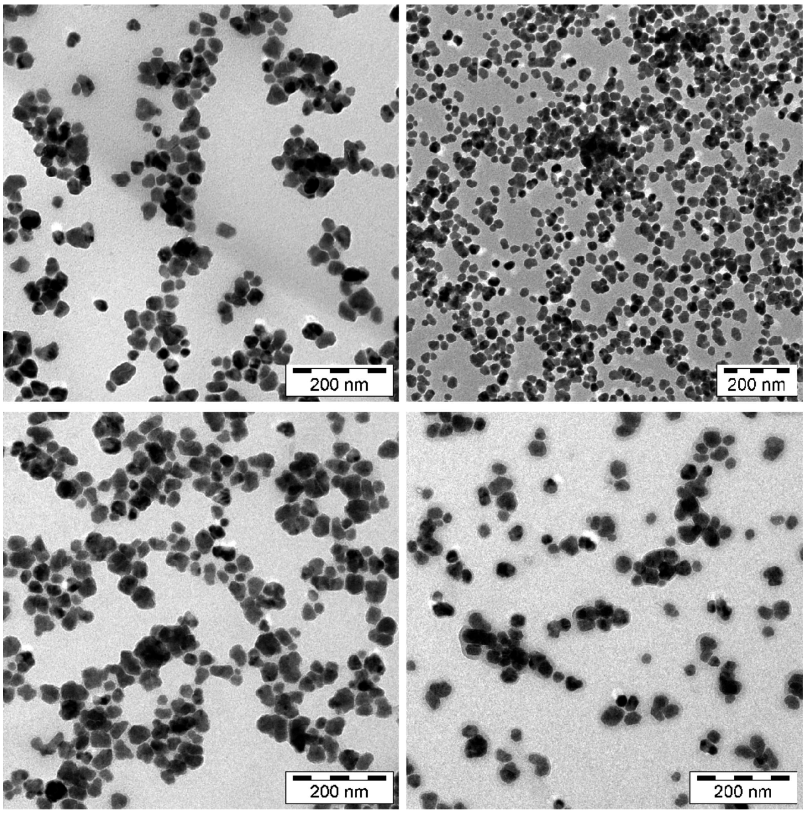

Figure 1. TEM pictures of the as-prepared nanocrystals: top left, $NaYbF_4$:Er; top right, $NaYbF_4$:Ho; bottom left, $NaYbF_4$:Tm; bottom right, $NaYF_4$:Yb.

sented in the Supporting Information. These particles should show additional peaks in the blue spectral range according to the literature, which can only be detected at higher temperature due to phonon-assisted processes or by means of higher excitation energies[12−14] and may be suitable for their use as a fifth color in further experiments. Thus, we are able to demonstrate the first four-color multiplexing by means of upconversion nanocrystals to date. Furthermore, the nanoparticles were encapsulated with SiO_2 by means of a microemulsion technique for phase transfer into aqueous dispersion or other polar solvents.

The upconversion mechanisms are shown in the Supporting Information, as well as the synthesis and the XRD patterns of the NCs prepared. Additionally, transmission electron microscopy (TEM) pictures and UC-PL spectra of the $NaYF_4$:Yb, Tb and $NaYbF_4$:Tb NCs are also shown there.

RESULTS AND DISCUSSION

The X-ray diffraction (XRD) patterns (Supporting Information) show the high crystallinity of the as-prepared (co)doped $NaYF_4$ and doped $NaYbF_4$ nanoparticles. A small amount of the cubic phase can be observed in the Yb-only system. This can be explained with the effective ionic radii of $Y^{3+} = 107.5$ pm and $Yb^{3+} = 104.2$ pm[15] for the coordination number of 9 because the smaller Yb ions can intercalate into the fluoride sublattice to form different crystalline structures. Also sodium ions can be placed on the RE^{3+} lattice sites with fluoride ions on in-between lattice sites.

The transmission electron microscope (TEM) micrographs show that the morphology can be described as dot- or potato-like as shown in Figure 1. The average particle size of any sample is measured to about 20 nm. The standard deviation of the TEM results can be explained by the decomposition reaction itself: the synthesis time is 30 min at 330 °C for $NaYbF_4$:RE and 5 min and 300 °C for the $NaYF_4$:Yb system. The resulting smaller particles here are obviously the building blocks of the bigger particles reported by Mai and co-workers. With the synthesis procedure presented here, the particle size of any of these particles can be standardized to 20 nm.

Figure 2 shows the IR-to-vis upconversion luminescence of the samples prepared in this work under 980 nm excitation. These pictures were recorded *without* the use of any optical filters, showing the four different colors, which can be separated either by the naked eye or spectrally (see below).

The infrared-to-visible upconversion spectra excited from the Yb^{3+} $^2F_{7/2}$ into the $^2F_{5/2}$ level with a 980 nm laser LED are shown in Figure 3. The typical upconversion levels in the presented systems can be observed: the $(^5F_4, ^5S_2)-^5I_8$ (540 nm) and $^5I_4-^5I_8$ (750 nm) transitions of Ho^{3+}, the $^1G_4-^3H_6$ (476 nm) and the $^3F_4-^3H_6$ (800 nm) transitions of Tm^{3+}, and the $^2H_{11/2}-^4I_{15/2}$ (520 nm), the $^4S_{3/2}-^4I_{15/2}$ (540 nm), and the $^4F_{9/2}-^4I_{15/2}$

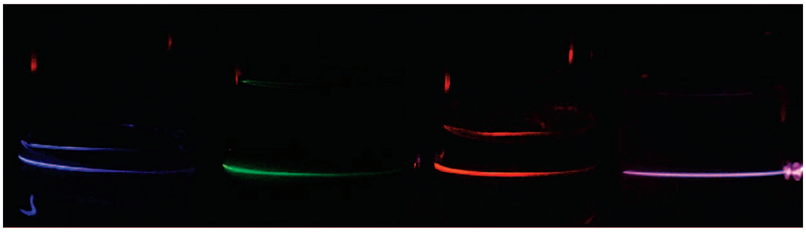

Figure 2. PL pictures of the as-prepared nanocrystals in chloroformic solutions, each excited with a 980 nm laser pointer (Roithner Lasertechnik, Vienna, Austria). From left to right, $NaYbF_4$:Tm, $NaYbF_4$:Ho, $NaYbF_4$:Er, and $NaYF_4$:Yb. These pictures were taken with a digital camera without the use of a red, green, or blue filter.

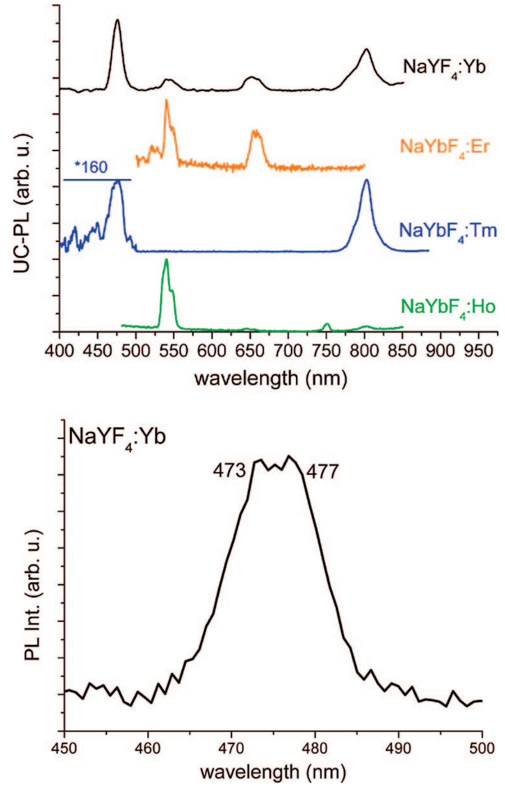

Figure 3. Upconversion spectra (top) of the four different kinds of NCs, excited with a 980 nm laser diode and zoomed view (bottom) on the 476 nm peak of the NaYF$_4$:Yb NCs.

(655 nm) of Er^{3+}. The 476 nm peak of the NaYF$_4$:Yb NCs cannot be explained by an overtone of the excitation source used; furthermore, no upconversion luminescence was observed in solution for pure NaYbF$_4$ NCs because of the sensitivity of the spectrometer used (data not shown). Therefore it has to be explained by the cooperative sensitization mechanism known from the Tm^{3+}-doped systems (note that Er, Ho, and Tm impurities are always present in purchasable Yb salts due to the production processes of the rare earth metals; these impurities can be seen in the UC-PL spectrum). This cooperative sensitization of pure Yb^{3+}-doped materials was also shown by Xiao *et al.* for Yb-doped barium gallogermanate glasses[16] (also showing the Er and Ho impurities) and by Maciel and co-workers for Yb-doped multicomponent silica glasses[17] (see Figure 3). The well-known upconversion processes are shown in Figure 4. Note that the ratio of the 476 and the 800 nm peak of the Tm system is about 1:160. With the peaks at 800, 655, and 750 nm, and the ratio of the 476/800 nm peaks, four differently colored labels can be separated in a multiplexing experiment as shown in Figure 5. The ratio of the 540 and the 655 nm peaks can be used to distinguish the Er from the Ho labels as well. To the best of our knowledge, this is the first four-color upconversion multiplexing experiment with nanocrystals to date. A five-color system is possible by the additional use of the Tb-doped particles.

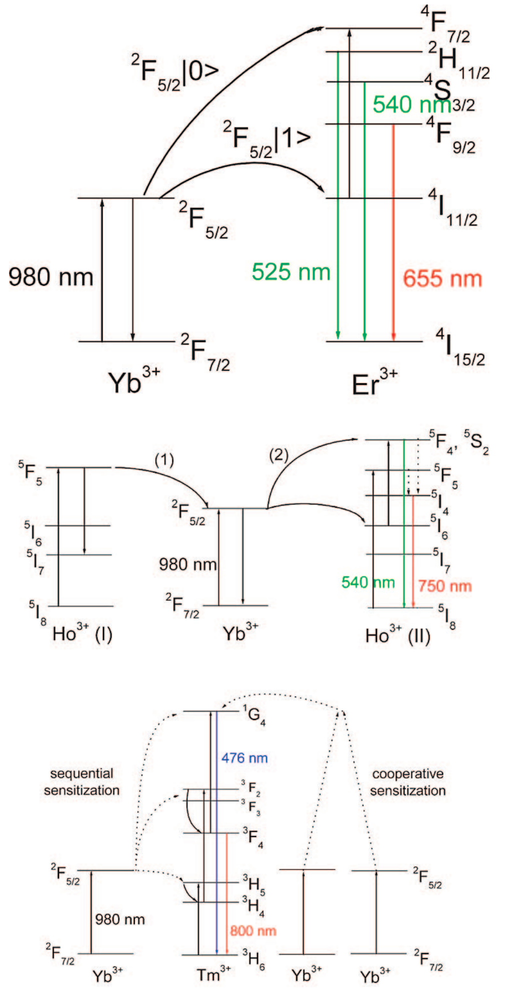

Figure 4. UC mechanisms of the Er- (top), Ho- (middle), and Tm-doped (bottom) NaYbF$_4$ nanocrystals. The cooperative upconversion mechanism of the Tm-doped particles also occurs in the NaYF$_4$:Yb system, leading to the cooperative luminescence without codopant.

The system NaY$_{1-x}$Yb$_x$F$_4$ with $x = 0.2$ is known to be the most effective upconversion host. Here, with $x = 1$, the particles presented in this work are slightly less effective than the ones with $x = 0.2$. However, using these particles, the spectral discernibility of the observed peaks is more effective and can even be observed by the naked eye.

Protecting nanocrystals by using different shelling materials can help to prevent the nanoparticles' degradation and enables further functionalization. Because the particles reported here cannot be degraded by hydrolysis or oxidation, we tried to encapsulate these particles with a silica shell. As an example, NaYbF$_4$:Tm NCs were encapsulated with a silica shell by means of a synthesis procedure similar to previously published methods of our group.[18−21] Briefly, Igepal CO-520 (Aldrich) was used instead of Synperonic NP-5 as capping agent in the microemulsion synthesis. The system NaYbF$_4$:Tm was chosen because of the most intense 800 nm peak in the systems described in this work and to exploit the

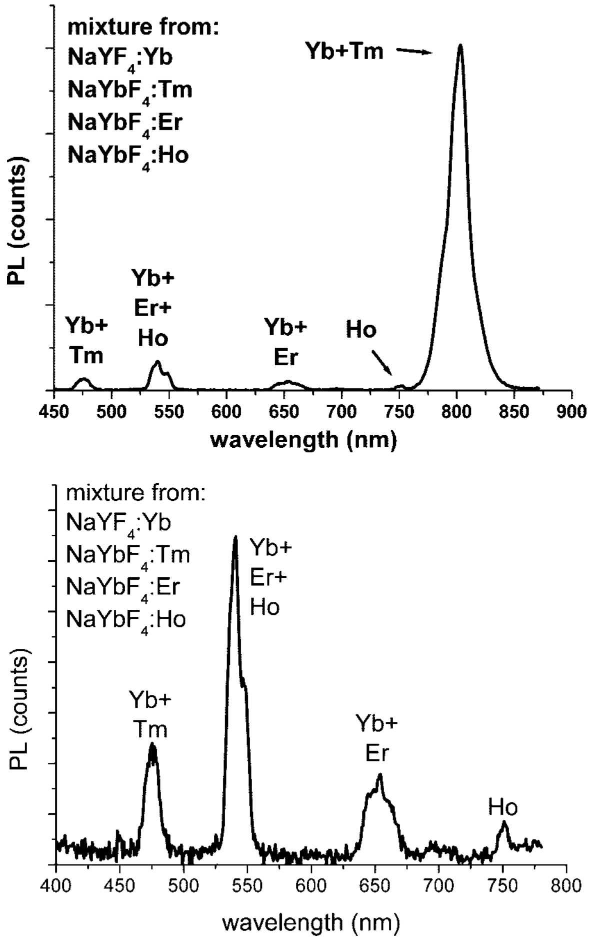

Figure 5. UC spectra with a mixture of the four different colors mentioned in this paper. As it can be seen, the four different colors can be spectrally separated under multiplexing conditions. Bottom figure zoomed for clarity.

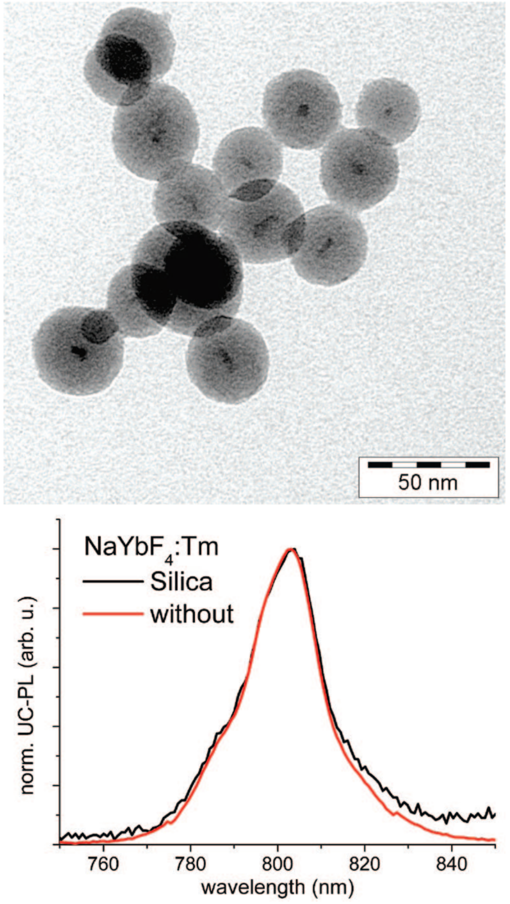

Figure 6. TEM micrograph (top) of NaYbF$_4$:Tm NCs encapsulated in silica spheres. As it can be seen, only one UC-NC is located in the center of the monodisperse silica spheres. UC-PL of the particles with (black) and without (red) silica encapsulation is shown in the bottom panel.

home-built spectral apparatus with the diluted solutions of the turbid silica dispersions.

The particles were successfully encapsulated with SiO_2 as shown in Figure 6. The TEM micrograph shows exactly one upconversion nanoparticle in the center of one silica sphere. The particles yielded are highly monodisperse and show the typical 800 nm peak position as the nonshelled ones. The silica encapsulation has no influence on the emission peak position as it is shown in Figure 5, because of the atomic transitions of the f elements used here, which are not influenced by the lattice phonons due to the geometry of the f orbitals (exception, the thermal line broadening). Only the absorbance spectra can be influenced by the ligands as decribed by Judd[22] and Ofelt.[23] The emission intensities before and after the encapsulation process were not investigated in detail because of the high concentrations of the silica dispersions needed to measure the spectra. These high concentrations lead to a too noisy background, which makes it impossible to record the absorbance spectra in the NIR region to compare the relative quantum yields. Even in the encapsulated spectrum in Figure 6 the scattering of the silica dispersion can be seen in that particular region.

With the use of these silica-encapsulated nanocrystals, covalent binding to marker reagents on the well-known SiO_2 surface is possible, for example, using $SiCl_4$ for activating the surface with subsequent coupling of these activated particles to linker or spacer molecules to prevent the biological markers from removal by physi- or chemisorped ligands, as is possible by the use of non-silica-shelled semiconductor nanocrystals like CdSe/ZnS or CdTe.

SUMMARY

We developed a new series of multicolor upconversion nanocrystals in the size range of biological relevance. The nanoparticles are highly crystalline, colloidal, and uniformly sized and show four different spectrally resolvable upconversion spectra. Mixtures of these particles were investigated under multiplexing conditions with spectral discernibility, which proves the usability of these particles for multiplexing analysis. With the use of the Tb-doped particles, a high-temperature five-color system is possible. The particles can be phase-transferred to polar solvents by SiO$_2$ encapsulation showing exactly one particle per silica sphere, enabling them for covalent coupling to marker molecules. The encapsulation has no influence on the upconversion properties. The coupling procedure and the use of the NCs for multicolor multiplexing applied to different bacterial pathogens in stool is currently under investigation.

METHODS

General Information. All chemicals were of analytical grade and used without further purification. Absorbance spectra were recorded on a TIDAS diode array spectrometer. The upconversion spectra were recorded by adapting a 980 nm laser diode (Roithner Lasertechnik, Vienna, Austria) and a home-built cuvette holder to the spectrometer. The detector was protected from the scattered excitation light by means of a 950 nm low-pass filter (Edmund Optics, Karlsruhe, Germany). The TEM images were taken using a Zeiss LEO 912 working at 120 kV.

Synthesis Procedure. The trifluoroacetates (TFA) of Yb, Tm, Er, Tb, and Ho were prepared by the procedure reported by Roberts.[24] The syntheses of the nanocrystals prepared in this work were inspired by the work of Mai *et al.*[11] The TFAs of Na (99%) and Y (99.99%), 1-octadecene (ODE, 95%), and oleic acid (OA, 90%) were purchased from Aldrich. In a typical procedure, 2.5 mmol of Na-TFA, 2.16 mmol of Yb-TFA, and 0.036 mmol of Tm-, Ho-, Tb-, or Er-TFA were dissolved at 100 °C in 6.5 mL of OA and 6.5 mL of ODE in a 100 mL three-necked flask with dust filter. For the synthesis of the NaYF$_4$:Yb and the NaYF$_4$:Yb, Tb particles, 1.8 mmol of Y-TFA and 0.36 mmol of Yb-TFA (+ 0.036 mmol Tb-TFA) were used instead of 2.2 mmol of Yb-TFA. The solution was degassed several times and purged with nitrogen. Afterward the solution was heated to 300 °C for 5 min (NaYF$_4$:Yb systems) or 330 °C for 30 min (NaYbF$_4$:Er, NaYbF$_4$:Ho, NaYbF$_4$:Tb, and NaYbF$_4$:Tm) to receive NCs of about 20 nm diameter. The flask was cooled down by means of compressed air, and the NCs were collected by addition of excess absolute ethanol and centrifugation. These NCs were dissolved in small amounts of heptane or toluene and additionally precipitated as described above. This procedure was repeated 3–4 times. Afterward the particles can easily be redispersed in chloroform, heptane, or toluene.

For the silica encapsulation, the solution of the NaYbF$_4$:Tm NCs was diluted to an absorbance value of 0.103 at 965 nm against pure chloroform; 500 μl of this solution and 1100 μl of Igepal CO-520 were used. The procedure is described elsewhere.[18–21]

Acknowledgment. The authors thank Dr. Martin Ade, Department of Inorganic and Analytical Chemistry, University of Freiburg, Germany, for the XRD measurements. The financial support of the German Ministry for Science and Education (Biophotonik II, UNDAM, Grant No. 13N8815) is gratefully acknowledged.

Supporting Information Available: XRD patterns of the as-prepared NaYF$_4$ and NaYbF$_4$ NCs, TEM and PL results of NaYF$_4$:Yb, Tb and NaYbF$_4$:Tb NCs, the corresponding Tb-based UC mechanisms, and a scheme of the home-built setup for the UC-PL measurements. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Riegler, J.; Ehlert, O.; Nann, T. A Facile Method for Coding and Labeling Assays on Polystyrene Beads with Differently Colored Luminescent Nanocrystals. *Anal. Bioanal. Chem.* **2006**, *384*, 645–650.
2. Riegler, J.; Nann, T. Application of Luminescent Nanocrystals as Labels for Biological Molecules. *Anal. Bioanal. Chem.* **2004**, *379*, 913–919.
3. Riegler, J.; Nick, P.; Kielmann, U.; Nann, T. Visualizing the Self-Assembly of Tubulin with Luminescent Nanorods. *J. Nanosci. Nanotechnol.* **2003**, *3*, 380–385.
4. Xu, S.; Kumar, S.; Nann, T. Rapid Synthesis of High Quality InP Nanocrystals. *J. Am. Chem. Soc.* **2006**, *128*, 1054–1055.
5. Alivisatos, A. P. Perspectives on the Physical Chemistry of Semiconductor Nanocrystals. *J. Phys. Chem.* **1996**, *100*, 13226–13239.
6. Ehlert, O.; Bücking, W.; Riegler, J.; Merkulov, A.; Nann, T. Organometallic Synthesis and Electrophoretic Characterization of High-Quality ZnS:Mn/ZnS Core/Shell Nanoparticles for Bioanalytical Applications *Microchim. Acta*, **2007**, DOI: 10.1007/s00604-007-0798-8.
7. Ehlert, O.; Osvet, A.; Batentschuk, M.; Winnacker, A.; Nann, T. Synthesis and Spectroscopic Investigations of Cu- and Pb-doped Colloidal ZnS Nanocrystals. *J. Phys. Chem. B* **2006**, *110*, 23175–23178.
8. Schäfer, H.; Ptacek, P.; Kömpke, K.; Haase, M. Lanthanide-Doped NaYF4 Nanocrystals in Aqueous Solution Displaying Strong Up-Conversion Emission. *Chem. Mater.* **2007**, *19*, 1396–1400.
9. Heer, S.; Kömpe, K.; Güdel, H.-U.; Haase, M. Highly Efficient Multicolour Upconversion Emission in Transparent Colloids of Lanthanide-doped NaYF4 Nanocrystals. *Adv. Mater.* **2004**, *16*, 2102–2105.
10. Auzel, F. Upconversion and Anti-Stokes Processes with f and d Ions in Solids. *Chem. Rev.* **2004**, *104*, 139–173.
11. Mai, H. X.; Zhang, Y. W.; Si, R.; Yan, Z. G.; Sun, L. D.; You, L. P.; Yan, C. H. High-Quality Sodium Rare-Earth Fluoride Nanocrystals: Controlled Synthesis and Optical Properties. *J. Am. Chem. Soc.* **2006**, *128*, 6426–6436.
12. Feng, L.; Zhang, J.; Wang, J.; Liang, H.; Su, Q. Cooperative Energy Transfer Frequency Upconversion in Tb3+/Yb3+-codoped Oxyfluoride Glasses. *Spectrochim. Acta, Part A* **2007**, *67*, 886–889.
13. Martins, E.; d. Araujo, C. B.; Delben, J. R.; Gomes, A. S. L.; d. Costa, B. J.; Messaddeq, Y. Cooperative Frequency Upconversion in Yb3+Tb3+ Codoped Fluoroindate Glass. *Opt. Commun.* **1998**, *158*, 61–64.
14. Menezes, L. d. S.; Maciel, G. S.; d. Araujo, C. B.; Messaddeq, Y. Phonon-Assisted Cooperative Energy Transfer and Frequency Upconversion in a Yb3+/Tb3+ Codoped Fluoroindate Glass. *J. Appl. Phys.* **2003**, *94*, 863–866.
15. Shannon, R. D. Revised Effective Ionic Radii and Systematic Studies of Interatomic Distances in Halides and Chalcogenides. *Acta Crystallogr.* **1976**, *A32*, 751–767.
16. Xiao, K.; Yang, Z. Blue Cooperative Luminescence in Yb3+-doped Barium Gallogermanate Glass Excited at 976 nm. *J. Fluoresc.* **2006**, *16*, 755–759.
17. Maciel, G. S.; Biswas, A.; Kapoor, R.; Prasad, P. N. Blue Cooperative Upconversion in Yb3+-doped Multicomponent Sol-Gel-Processed Silica Glass for Three-Dimensional Display. *Appl. Phys. Lett.* **2000**, *76*, 1978–1980.
18. Darbandi, M.; Lu, W.; Fang, J.; Nann, T. Silica Encapsulation of Hydrophobically Ligated PbSe Nanocrystals. *Langmuir* **2006**, *22*, 4371–4375.
19. Darbandi, M.; Nann, T. One-Pot Synthesis of YF3@Silica Core/Shell-Nanoparticles. *Chem. Commun.* **2006**, 776–778.
20. Darbandi, M.; Thomann, R.; Nann, T. Single Quantum Dots in Silica Spheres by Microemulsion Synthesis. *Chem. Mater.* **2005**, *17*, 5720–5725.
21. Nann, T.; Mulvaney, P. Single Quantum Dots in Spherical Silica Particles. *Angew. Chem., Int. Ed.* **2004**, *43*, 5393–5396.
22. Judd, B. R. Optical Absorption Intensities of Rare-Earth Ions. *Phys. Rev.* **1962**, *127*, 750–761.
23. Ofelt, G. S. Intensities of Crystal Spectra of Rare-Earth Ions. *J. Chem. Phys.* **1962**, *37*, 511–520.
24. Roberts, J. E. Lanthanum and Neodymium Salts of Trifluoroacetic Acid. *J. Am. Chem. Soc.* **1961**, *83*, 1087–1088.