

**Fluorescent Labels**

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**Monodisperse Silica-Coated Polyvinylpyrrolidone/NaYF<sub>4</sub> Nanocrystals with Multicolor Upconversion Fluorescence Emission\*\***

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Nanosized fluorescent labeling materials have been widely used for biological studies and clinical applications. Conventional downconversion fluorescent labels require an ultra-violet or blue excitation wavelength.<sup>[1,2]</sup> These single-photon

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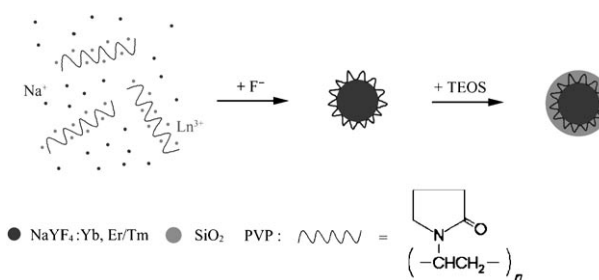
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fluorescent labels emit one lower-energy photon after absorbing a higher-energy UV or visible photon. Their disadvantages include low light-penetration depth and possible severe photodamage to living organisms. Furthermore, many biological samples show autofluorescence under short-wavelength UV radiation, which decreases the sensitivity of detection. It is desirable to use fluorescent labels that can be excited in the near-infrared (NIR) region.<sup>[3]</sup> The IR-to-visible upconversion nanocrystal is a good choice.<sup>[4,5]</sup> The nanocrystal emits one higher-energy photon after absorbing two or more lower-energy photons. Different colors of visible light can be obtained from different upconversion phosphors when excited by the same IR laser.<sup>[4]</sup> In comparison with downconversion fluorescent materials, upconversion nanocrystals show very low background light as a consequence of their unique fluorescent properties. In addition, photodamage to biological tissues is minimal because the tissues are usually transparent to NIR light.<sup>[6]</sup> NaYF<sub>4</sub> has been reported as the most efficient host material for Yb/Er (or Yb/Tm) co-doped IR-to-visible upconversion phosphors.<sup>[7]</sup> Colloidal NaYF<sub>4</sub> nanocrystals co-doped with Yb/Er and Yb/Tm have been prepared, with strong upconversion fluorescence seven orders of magnitude higher than that of CdSe–ZnS quantum dots.<sup>[8,9]</sup>

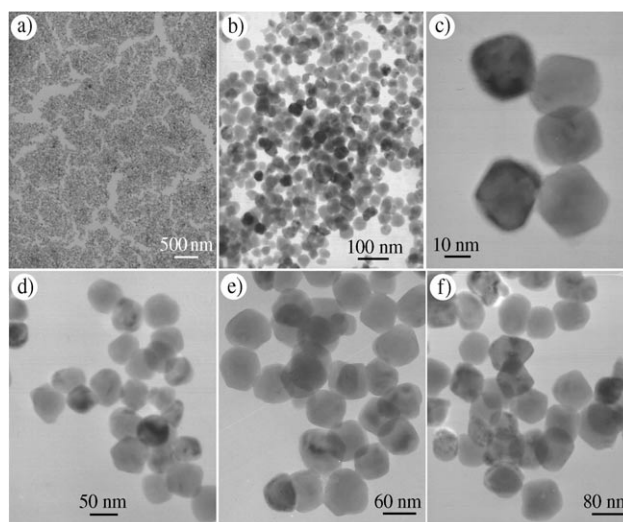
Some efforts have been made to produce upconversion NaYF<sub>4</sub> nanocrystals with controlled size and shape.<sup>[5,8,10]</sup> Ethylenediaminetetraacetic acid (EDTA) was used as a chelating agent to control the growth of NaYF<sub>4</sub> nanocrystals, but the as-prepared nanocrystals tended to precipitate in solution as a result of the lack of both hydrophilic and hydrophobic chemical groups on their surface.<sup>[5,10]</sup> Colloidal solutions of NaYF<sub>4</sub> nanocrystals were prepared; however, toxic chemicals and laborious procedures were used. Furthermore, these nanocrystals were hydrophobic and could only be dispersed in certain organic solvents such as hexane and dimethyl sulfoxide (DMSO) under ultrasound sonication.<sup>[8,11,12]</sup> The use of these nanocrystals directly for biological applications is limited because of their very low solubility in water and unsuitable surface property. It is necessary to develop suitable methods for synthesizing upconversion NaYF<sub>4</sub> nanocrystals that are dispersible in water and many organic solvents, and also have some functional chemical groups on their surfaces for conjugation of biomolecules.<sup>[9]</sup>

The selection of a suitable chelating agent for controlling the growth of the nanocrystals and of a surfactant to stabilize the nanocrystals and provide them with a desirable surface is the key to solving the above-mentioned problems. Polyvinylpyrrolidone (PVP) is an amphiphilic surfactant which can render the nanocrystals dispersible in water and organic solvents.<sup>[13]</sup> Furthermore, its pyrrolidone groups can coordinate with lanthanide ions.<sup>[14,15]</sup> As such it was used in this work as both the chelating agent and stabilizer to synthesize NaYF<sub>4</sub> nanocrystals with controlled size and shape, and with suitable surface property and solubility in water (Figure 1). The PVP-stabilized NaYF<sub>4</sub> nanocrystals could be directly coated with a uniform layer of silica to produce a surface for the conjugation of biomolecules.

Figure 2 shows TEM images of the PVP/NaYF<sub>4</sub>:Yb,Er nanocrystals. The crystals are polyhedral in shape and very uniform in size with an average size of about 30 nm



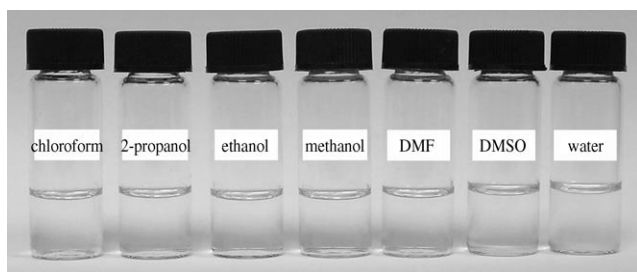
**Figure 1.** Synthesis of silica-coated PVP/NaYF<sub>4</sub> nanocrystals doped with lanthanide ions. TEOS = tetraethoxysilane.



**Figure 2.** a–c) TEM images with different resolutions of PVP/NaYF<sub>4</sub>:Yb,Er nanocrystals with a size of 30 nm. d–f) TEM images of nanocrystals with different sizes: d) 48; e) 65; f) 87 nm.

(Figure 2a–c). The black spots in the images result from PVP adsorbed on the surface of the nanocrystal, which was burnt out under exposure to the accelerated electron beam during the TEM measurement. The sizes of the PVP/NaYF<sub>4</sub> nanocrystals could be altered by changing the experimental conditions, such as concentrations of the reactants, and reaction temperature and time. The sizes could be adjusted within the range of 20–100 nm with a relatively narrow size distribution (Figure 2d–f). The XRD pattern of the nanocrystals (Figure S1 in the Supporting Information) agrees well with the data for pure cubic NaYF<sub>4</sub> nanocrystals, as reported in the JCPDS card (No. 77-2042,  $a = 5.470 \text{ \AA}$ ), thus indicating a high purity of the NaYF<sub>4</sub> nanocrystals obtained. It was previously reported that in most cases a mixture of cubic and hexagonal NaYF<sub>4</sub> crystals was produced, although several methods for synthesizing pure cubic and hexagonal crystals have been developed.<sup>[6,8,16]</sup> The method presented herein seems to be efficient for producing pure-phase NaYF<sub>4</sub> nanocrystals in solution. The FTIR spectrum of the nanocrystals (Figure S2 in the Supporting Information) shows a peak at  $1640 \text{ cm}^{-1}$  that could be assigned to the C=O stretching vibration, which indicates the presence of PVP on the surface of the nanocrystals.

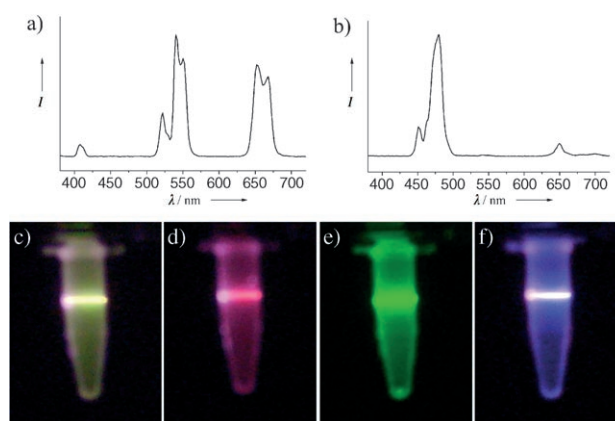
It has been demonstrated that only microsized  $\text{NaYF}_4$  crystals can be formed if water is used as the solvent and no chelating agents are employed.<sup>[5,10]</sup> The strong chelating agent EDTA was used to form a complex with lanthanide ions to control the growth of the crystals and their size. According to LaMer's model, the formation of such a complex could control the concentration of lanthanide ions in the solution, and thus help to control the nucleation and growth of the crystals. In this work, lanthanide ions were complexed with the pyrrolidone groups of PVP and then released slowly to the solution to react with fluoride ions in the viscous and weakly polar solvent ethylene glycol, and thus small nanocrystals were formed.<sup>[14,15]</sup> Furthermore, PVP could also serve as a stabilizer of the nanocrystals.<sup>[17]</sup> As a result of the good solubility of PVP in water and many organic solvents, it is expected that the PVP/ $\text{NaYF}_4$  nanocrystals can be well-dispersed in water and many organic solvents to form colloidal solutions. Especially, the nanocrystals should be dispersible in water and some polar solvents such as ethanol, otherwise their use in bio-related applications is very limited. The photograph in Figure 3 demonstrates that the PVP-



**Figure 3.** Photographs of colloidal solutions of the PVP/ $\text{NaYF}_4$ :Yb,Er nanocrystals dispersed in different solvents. DMF = *N,N*-dimethylformamide.

stabilized  $\text{NaYF}_4$  nanocrystals are well-dispersed in some of the most commonly used solvents, from weakly polar chloroform to strongly polar water, to form transparent colloidal solutions. As for nonpolar solvents such as hexane, the solubility of the nanocrystals was about eight times lower than that in polar solvents. However, the nanocrystals could still be dispersed in a mixture of polar and nonpolar solvents, for example, a mixture of equal amounts of hexane and ethanol.

Fluorescence spectra of the lanthanide-doped PVP/ $\text{NaYF}_4$  nanocrystals in ethanol solution are shown in Figure 4. The emission peaks of the PVP/ $\text{NaYF}_4$ :Yb,Er nanocrystals at 407, 521, 539, and 651 nm were a result of the transitions from  $^4\text{H}_{9/2}$ ,  $^4\text{H}_{11/2}$ ,  $^4\text{S}_{3/2}$ , and  $^4\text{F}_{9/2}$  to  $^4\text{I}_{15/2}$  of  $\text{Er}^{3+}$ , respectively. The emission peaks of the PVP/ $\text{NaYF}_4$ :Yb,Tm nanocrystals at 450, 479, and 649 nm were assigned to the  $^1\text{D}_2 \rightarrow ^3\text{F}_4$ ,  $^1\text{G}_4 \rightarrow ^3\text{H}_6$ , and  $^1\text{G}_4 \rightarrow ^3\text{F}_4$  transitions of  $\text{Tm}^{3+}$ .<sup>[6]</sup> It is well-known that the crystallinity and surface property of the nanocrystals could alter the intensity of the fluorescence emission peaks of the doping lanthanide ions. For the  $\text{NaYF}_4$  nanocrystals prepared using the co-precipitation method, the intensity of the green fluorescence emission peak is much higher than that of the red fluorescence emission peak,<sup>[5,10]</sup> while the emission peak of red fluorescence is much stronger

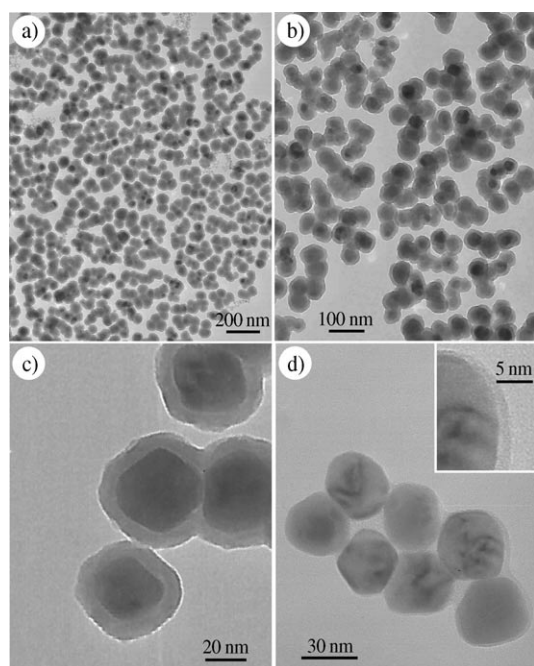


**Figure 4.** Fluorescence spectra of colloidal solutions of a) Yb/Er-doped and b) Yb/Tm-doped PVP/ $\text{NaYF}_4$  nanocrystals (30 nm) in ethanol. c–f) Photographs of the PVP/ $\text{NaYF}_4$ :Yb,Er nanocrystals showing total fluorescence (c), fluorescence passing through filters (d, e) for red and green light, respectively, and PVP/ $\text{NaYF}_4$ :Yb,Tm nanocrystals (f), under excitation from a NIR laser (980 nm).

than the peak of green fluorescence for the nanocrystals stabilized by oleic acid.<sup>[12]</sup> For the PVP/ $\text{NaYF}_4$ :Yb,Er nanocrystals, strong green and red fluorescence were observed, which suggests that either green or red fluorescence can be seen selectively by using suitable filters for green or red light. Strong fluorescence with different colors from the PVP/ $\text{NaYF}_4$  nanocrystals co-doped with Yb/Er or Yb/Tm can be seen under the excitation of a 980-nm NIR laser (see Figure 4c–f). The color of the colloidal solution of PVP/ $\text{NaYF}_4$  nanocrystals could be tuned by mixing the nanocrystals co-doped with Yb/Er and Yb/Tm at certain ratios.

Besides the good solubility in water and organic solvents, the PVP/ $\text{NaYF}_4$  nanocrystals could be coated with silica directly, whereas other  $\text{NaYF}_4$  nanocrystals without PVP generally required some surface modifications before coating.<sup>[18,19]</sup> The silica coating can improve the photostability and biocompatibility of the nanocrystals, and the protocol for conjugation of biomolecules to a silica surface is well established.<sup>[20–22]</sup> TEM images of the silica-coated PVP/ $\text{NaYF}_4$ :Yb,Er nanocrystals are presented in Figure 5. For the first time, we have demonstrated the formation of core-shell-structured silica/PVP/ $\text{NaYF}_4$  nanocrystals with a very uniform layer of silica on the  $\text{NaYF}_4$  nanocrystals. The TEM images showed that the thickness of the silica shell was about 10 nm and could be adjusted to 1–3 nm by adding a smaller amount of TEOS. After coating with silica, the nanocrystals can still emit strong upconversion fluorescence and are quite stable in water (Figure S3 in the Supporting Information).

In summary, ultrafine, pure cubic PVP/ $\text{NaYF}_4$  nanocrystals co-doped with lanthanide ions such as Yb/Er or Yb/Tm were synthesized using PVP as a chelating agent and stabilizer. The nanocrystals are very uniform and the size is tunable by adjusting the experimental conditions. Furthermore, the nanocrystals are monodisperse in water and some of the most commonly used organic solvents, and strong multicolor upconversion fluorescence was observed from the clear colloidal solution of the nanocrystals under excitation from a 980-nm NIR laser. Moreover, a uniform layer of silica



**Figure 5.** a–c) TEM images of silica-coated PVP/NaYF<sub>4</sub>:Yb,Er nanocrystals. d) TEM image of PVP/NaYF<sub>4</sub>:Yb,Er nanocrystals coated with a very thin silica layer.

was coated onto the PVP/NaYF<sub>4</sub> nanocrystals to form a core-shell structure, with the silica thickness adjustable down to 1–3 nm. These upconversion nanocrystals have great potential for use as a fluorescent labeling material for biological applications.

### Experimental Section

All the chemical reagents were purchased from Sigma-Aldrich and used without further purification. The PVP/NaYF<sub>4</sub>:Yb(20%),Er(2%) nanocrystals were synthesized as follows. Y<sub>2</sub>O<sub>3</sub> (88 mg, 0.78 mmol), Yb<sub>2</sub>O<sub>3</sub> (39.4 mg, 0.2 mmol), and Er<sub>2</sub>O<sub>3</sub> (3.9 mg, 0.02 mmol) were dissolved in 10% HNO<sub>3</sub> (10 mL), and then the solution was heated to evaporate the water completely. Ethylene glycol (10 mL) was added to dissolve the obtained LnNO<sub>3</sub> (Ln = Y 78%, Yb 20%, Er 2%). Polyvinylpyrrolidone (PVP 40, 0.5560 g) and NaCl (0.0588 g, 1 mmol) were subsequently added and the solution was heated to 80 °C until a homogeneous solution was formed. NH<sub>4</sub>F (0.1482 g, 4 mmol) was dissolved in ethylene glycol (10 mL) at 80 °C and added dropwise to the LnNO<sub>3</sub> solution, which was maintained at 80 °C for 10 min under stirring. The solution was heated to 160 °C for 2 h and then cooled to room temperature. The product was isolated by centrifugation and washed twice with absolute ethanol. The PVP/NaYF<sub>4</sub>:Yb(20%),Tm(2%) nanocrystals were synthesized using the same protocol except that Er<sub>2</sub>O<sub>3</sub> was replaced by Tm<sub>2</sub>O<sub>3</sub>.

A typical procedure for coating silica onto the PVP/NaYF<sub>4</sub> nanocrystals was as follows. PVP/NaYF<sub>4</sub>:Yb(20%),Er(2%) nanocrystals (0.05 mmol) were dispersed in ethanol (20 mL) and mixed with water (4 mL) and ammonia (30%, 0.5 mL). Tetraethoxysilane (TEOS, 0.06 mL) dissolved in ethanol (10 mL) was then added slowly to the solution with continuous stirring. The product was isolated by centrifugation and washed twice with water.

The samples were also studied with a JEOL 2010F transmission electron microscope. Fluorescence spectra were recorded on a Hitachi F-500 fluorescence spectrophotometer and fluorescence

images were captured using an Axiostar plus fluorescence microscope, equipped with a NIR laser (980 nm).

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