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**NaGdF₄:Eu³⁺ Nanoparticles for Enhanced X-ray Excited Optical Imaging**

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**ABSTRACT:** X-ray luminescent nanoparticles (NPs), including lanthanide fluorides, have been evaluated for application to deep tissue in vivo molecular imaging using optical tomography. A combination of high material density, higher atomic number and efficient NIR luminescence from compatible lanthanide dopant ions indicates that particles that consist of Al₅F₄ (A = alkaline, Ln = lanthanide element) may offer a very attractive class of materials for high resolution, deep tissue imaging with X-ray excitation. NaGdF₄:Eu³⁺ NPs produced an X-ray excited luminescence that was among the most efficient of nanomaterials that have been studied thus far. We have systematically studied factors such as (a) the crystal structure that changes the lattice environment of the doped Eu³⁺ ions within the unit cell; and extrinsic factors such as (b) a gold coating (with attendant biocompatibility) that couples to a plasmonic excitation, and (c) changes in the NPs surface properties via changes in the pH of the suspending medium—all with a significant impact on the X-ray excited luminescence of NaGdF₄:Eu³⁺NPs. The luminescence from an optimally doped hexagonal phase NaGdF₄:Eu³⁺ nanoparticle was 25% more intense compared to that of a cubic structure. We observed evidence of plasmonic reabsorption of midwavelength emission by a gold coating on hexagonal NaGdF₄:Eu³⁺ NPs; fortunately, the NaGdF₄:Eu³⁺ @Au core–shell NPs retained the efficient ⁵D₀→⁷F₄ NIR (692 nm) luminescence. The NaGdF₄:Eu³⁺ NPs exhibited sensitivity to the ambient pH when excited by X-rays, an effect not seen with UV excitation. The sensitivity to the local environment can be understood in terms of the sensitivity of the excitons that are generated by the high energy X-rays (and not by UV photons) to crystal structure and to the surface state of the particles.

**INTRODUCTION**

Scintillation materials that emit visible photons when irradiated with γ- or X-rays constitute an extreme form of optical down-conversion. Lanthanides as dopants are often a good choice for such materials, as they have high atomic number and suitable electronic energy states to emit photons in the UV, visible, and near-infrared (NIR) region of the electromagnetic spectrum. Elements with a high atomic number in a host matrix generate sufficiently high-energy electrons that can be down-converted in energy by an appropriate choice of lanthanide sensitizers and emitters. Lanthanide-doped nanophosphors, especially up-converting nanophosphors, are increasingly finding application in vitro and in vivo imaging. Recently, nanophosphors have been explored as agents in radiological applications, especially in vivo tumor detection. The combination of insignificant scattering of X-rays in tissues, and the high tissue penetration of NIR optical photons emitted from appropriate phosphors, opens up the possibility of achieving deep tissue optical imaging in vivo with unprecedented spatial resolution. As X-ray scattering in tissues is negligible, optical imaging is only restricted by the width of the X-ray beam. Because the X-ray excitation path is well-defined, an accurate reconstruction of the target region of interest with a high degree of spatial resolution is possible by employing X-ray luminescence optical tomography (XLOT). Even with the relatively small number of photons that can be generated from optically emitting nanoparticles following excitation by a standard X-ray source, a good signal-to-noise ratio for reconstruction of the luminescent emission region can be obtained, in which the optical signal is proportional to the X-ray luminescent particle concentration. Using this approach, in vitro imaging of nanoparticles in tissue phantoms has already been demonstrated for metal oxides (Gd₂O₃:Eu) and for oxysulfides(Gd₂O₂S:Eu). New materials with enhanced luminescence properties are very desirable for XLOT applications. Although it has been shown that low bandgap materials (e.g., ZnS:Ag) offer a greater...
efficiency as scintillator materials, fluoride materials offer other advantages in terms of high density, mechanical hardness, and radiation hardness. A fluoride matrix offers efficient transfer pathways for self-trapped excitons (STE) to the activator ions. Therefore, selecting a rare-earth activator promotes tuning of the emission upon X-ray excitation, which opens up new possibilities for in vivo applications for nanoparticles using these activated fluoride materials. As an example, BaYF<sub>5</sub>:Eu<sup>3+</sup> nanoparticles (15–20 nm) have recently been shown to be efficient markers for in vivo radioluminescence imaging.

Fluoride-based AlLnF<sub>4</sub> (A = alkaline; Ln = lanthanide) materials are preferred hosts for optically emitting lanthanide phosphors since they have low phonon energy states. AlLnF<sub>4</sub> is the preferred matrix for down-conversion, as it is a wide band gap material with a band gap in the range 9–10 eV. The rare-earth dopant causes a contraction of the host material that results in unit-cell densities of as high as 8.44 g/cm<sup>3</sup> with attendant increase in X-ray absorption. In addition, rare-earth doping can serve as both an activator and sensitizer center. With the combined favorable properties that arise from rare-earth doping, improved materials for XLOT imaging applications may be identified.

Gadolinium serves as an excellent photosensitizer for UV-excited down-converting lanthanide emitters. The Gd<sup>3+</sup>–Eu<sup>3+</sup> host–dopant combination is the best-studied down-converting system with lower energy UV radiation because the emission energy transitions within Gd<sup>3+</sup> can resonantly couple to the excited state of Eu<sup>3+</sup> ions. Based on this established science, AGdF<sub>4</sub>:Eu<sup>3+</sup> nanoparticles appear to be a very good choice for XLOT, as this material could provide an efficient means to down-convert high energy electrons generated by X-rays to visible or near-infrared (NIR) wavelengths. The efficiency of scintillators in bulk materials is well-documented, but a systematic comparison of the efficiency of optical emission by doped fluoride nanoparticles (as well as other doped metal oxide nanoparticles), and their sensitivity to surface states, is not available to the best of our knowledge.

Nanoparticles can be modified by adding coatings to their surfaces to improve the absorption of X-rays. Phosphor materials typically offer maximum densities in the range of 10 g/cm<sup>3</sup>. Metals, on the other hand, have densities of ~20 g/cm<sup>3</sup>. Therefore, in order to improve the X-ray stopping power of the phosphor material, a core–shell structure consisting of a phosphor core and metal shell may offer some advantages. The addition of a gold shell to the phosphors can contribute to the biocompatibility to the nanoparticles, as well as potentially improve the efficiency of the X-ray luminescence. We previously developed a synthesis route for the addition of a gold shell to up-converting phosphors, which yields a significant increase in the efficiency of the up-conversion process. This core–shell material was chosen as a possibly interesting candidate for clinical applications of the XLOT technology and has been examined in the current study, along with bare phosphor particles. Most of the efforts over many years in developing phosphors for scintillator applications have been devoted to examining bulk and single crystals. The use of these materials in a nanoparticle form stems from the need for in vitro and in vivo imaging methodologies. Currently, very little information pertaining to intrinsic and extrinsic factors influencing the X-ray excited luminescence is available for X-ray excitable nanophosphors that are less than 100 nm in size. Here, a systematic study of NaGdF<sub>4</sub>:Eu<sup>3+</sup> nanoparticles has been undertaken with the purpose of developing a well controlled nanomaterial platform (in terms of uniformity, size, tunability, and surface modification) for X-ray excited optical imaging with a good understanding of the factors that control the luminescence. The comparison of X-ray excited luminescence of NaGdF<sub>4</sub>:Eu<sup>3+</sup> with other reported materials of a similar size show that NaGdF<sub>4</sub>:Eu<sup>3+</sup> nanoparticles exhibit the greatest X-ray excited luminescence. Therefore, in addition to their potential as an efficient magnetic resonance imaging (MRI) contrast agent and up-converting phosphor, this class of nanomaterial holds the great promise as a new XLOT imaging agent.

## EXPERIMENTAL SECTION

### Synthesis of NaGdF<sub>4</sub>-Doped with Eu<sup>3+</sup>

Synthesis of NaGdF<sub>4</sub>-doped with Eu<sup>3+</sup>. Nanoparticles of NaGdF<sub>4</sub>-doped with Eu<sup>3+</sup> and Ce<sup>3+</sup> were synthesized by the citrate method. A transparent aqueous solution containing 4 mL of 0.2 M lanthanide chlorides (Sigma Aldrich, MO) and 8 mL of 0.2 M sodium citrate (Sigma Aldrich, MO) was heated to 90 °C. Sodium fluoride (16 mL of 1 M) (Fischer Scientific, PA) solution was added to the solution, upon which the solution turned whitish. The nanoparticles were heated for 2 h. The nanoparticles were centrifuged and washed twice before further measurements were carried out. As-synthesized particles were heated in a Teflon-lined autoclave (Cole-Parmer, IL) at 210 °C for 1 h to obtain hexagonal phase of the nanoparticles. The pH studies were carried out by suspending 6 mg, 2.5 mg, and 2 mg of the nanoparticles obtained from three different batches in 1 mL of phosphate buffer silane with pH values ranging from 2.5 to 7.2. pH buffers below 7.4 were prepared by adding small amounts of 10 mM solution of HCl was used and the pH monitored by ORION 5 STAR (Thermo Scientific, MA) pH meter.

### Synthesis of BaYF<sub>5</sub>:Eu<sup>3+</sup> in Hydrothermal Method.

Water-soluble and polyethyl glycol (PEG)-coated BaGdF<sub>5</sub>:Eu<sup>3+</sup> nanoparticles were synthesized following a reported one-pot hydrothermal method. Then, 0.90 mmol of GdCl<sub>3</sub>·6H<sub>2</sub>O and 0.10 mmol of EuCl<sub>3</sub>·6H<sub>2</sub>O were added to 20 mL ethylene glycol (EG). Then, 1 mmol BaCl<sub>2</sub>·2H<sub>2</sub>O was added to above solution and stirred for 30 min. PEG (1 g) (M<sub>c</sub> = 2000) was added and sonicated (Branson 2510, Branson Ultrasonic Cleaner) the solution for 15 min. After that, 10 mL of EG containing 5.5 mmol NH<sub>4</sub>F was added to above mixture. The mixture solutions were stirred and sonicated for another 30 min and then transferred into a 50 mL stainless Teflon-lined autoclave and kept at 200 °C for 24 h. After that, the autoclave was allowed to cool down to room temperature. The reaction mixture was washed and centrifuged several times with ethanol and deionized (DI) water to remove other residual solvents and then suspended in DI water for further use.

### Synthesis of BaYF<sub>5</sub>:Eu<sup>3+</sup> by the Thermal Decomposition Method.

Hydrophobic nanocrystals were synthesized via a thermal decomposition method. YCl<sub>3</sub>·6H<sub>2</sub>O (0.90 mmol), 0.10 mmol of EuCl<sub>3</sub>·6H<sub>2</sub>O, and 1 mmol of barium acetylacetonate were dissolved in 15 mL oleic acid and 15 mL of 1-octadecene at 120 °C under vacuum for 2 h. The mixture was then cooled to room temperature, and a second solution containing 5.5 mmol of NH<sub>4</sub>F, in 10 mL methanol was added. The mixture was stirred for 30 min and the methanol was slowly removed at 70 °C. Once the methanol was removed the reaction mixture was raised to 310 °C and heated for 1 h. The solution was cooled to room temperature and washed with ethanol and oleate capped nanoparticles were then dispersed in chloroform. The hydrophobic nanoparticles were rendered water-dispersible by intercalating with PEG-mono oleate ligands (Sigma, M<sub>n</sub> ~ 860) using a method described elsewhere. The PEG-modified nanoparticles then were dispersed in DI water for further use.

### Synthesis of Gd<sub>2</sub>O<sub>3</sub>:Eu<sup>3+</sup>

Nanoparticles were synthesized using an ultrasonic flame spray pyrolysis technique similar to the forced jet atomizer method reported by our group earlier. Briefly, 35.5 mM of Gd(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O and 6.3 mM of Eu(NO<sub>3</sub>)<sub>3</sub>·6H<sub>2</sub>O salts were dissolved in ethanol and used as the liquid precursor. The precursor was atomized at 50 mL/h flow using a tunable ultrasonic
spray nozzle (Sono-Tek Corporation, NY, U.S.A.) operating at 1.9 W with a nitrogen carrying gas (3 L/min) into a hydrogen (2 L/min)–air flame. The precursor droplets were pyrolyzed to form nanoparticles in the high temperature environment. Particles were collected on a 200 nm PTFE membrane filter; the collected powder was washed in Milli-Q ultrapure (MQ, 18.3 MΩ cm) water to remove any unreacted precursor from the nanoparticles. The nanoparticles were dispersed in DI water for further characterization.

**Powder X-ray Diffraction.** The phases of the calcined powders of coated and uncoated NPs were characterized by XRD. X-ray tube (SB80250, Oxford Instruments, Scott Valley, CA), with a copper Kα source, operating at 45 KVP and 40 mA. Powdered samples were spread on a low background glass slide and scanned in a 2θ–20° geometry from 10 to 60 degrees.

**Transmission Electron Microscopy.** The nanoparticles were deposited on a Formvar/carbon coated copper grids from a water solution and dried before taking the bright field transmission electron micrographs from Phillips CM-12 operating at 100–120 KeV and 10–15 μA.

**Dynamic Light Scattering Experiments.** A 0.1% solution of these core–shell nanoparticles was prepared in water. The dynamic light scattering experiments were performed with the Brookhaven ZetaPlus instrument. A single run was of 2 min duration and the data was averaged over five runs for each sample.

**Absorbance.** The optical absorbance of nanoparticle solutions was obtained from a spectrophotometric method using a Varian Cary 5000 instrument for different types of particles at 30 mg/mL concentration. Each sample was sonicated and 25 μL of it was mixed with 25 μL of the nanoparticles in a cuvette. The typical particle size of the hexagonal NaGdF₄:Eu³⁺ nanoparticles was approximately 30 nm (Figure 1a). In Figure 1b, the integrated intensities of the three major ⁵D₀→⁷F₁ transitions observed. In Figure 1c, X-ray luminescence spectra of cubic and hexagonal NaGdF₄:15%Eu³⁺ nanoparticles. Nanoparticle concentration in all the luminescence measurements was 3 mg/mL.

**RESULTS AND DISCUSSION**

The typical particle size of the hexagonal NaGdF₄:Eu³⁺ nanoparticles was approximately 30 nm (Figure 1a). In Figure 1b, the integrated intensities of the three major ⁵D₀→⁷F₁ emissions of Eu³⁺ are plotted against the molar percentage of the Eu³⁺ in the nanoparticles. The intensities increased with Eu³⁺ concentration and reached a maximum at about 15 molar percent concentration of Eu³⁺ in hexagonal NaGdF₄ nanoparticles. A similar concentration trend was observed for cubic NaGdF₄ nanoparticles, albeit with half the intensities.
of the emissions under X-ray excitation are shown in Figure 1c. The X-ray luminescence optical spectrum was obtained from a solution of nanoparticles with a concentration of 3 mg/mL. As XLOT requires bright luminescent particles, optimization of the dopant concentration is critical for efficient imaging. However, to the best of our knowledge, there are no extant reports with respect to the optimized doping concentration of Eu³⁺ for X-ray luminescence. A comparison of X-ray activated optical luminescence spectra from hexagonal and cubic NaGdF₄:Eu³⁺ reveals that the emission peaks are identical in the 300–1100 nm region (Figure 1c). There was no change in the ratio of intensities of the emission peaks that arise from the magnetic dipole and electric dipole transitions, ⁵D₀→⁷F₁ (587 nm) and ⁵D₀→⁷F₂ (612 nm), respectively. The ratio of ⁵D₀→⁷F₁/⁵D₀→⁷F₂ was 1.6 and 1.7 for cubic and hexagonal crystals. This indicates the X-ray luminescence was from a highly asymmetric Eu³⁺ for both the structures under X-ray excitation.

The trend of the X-ray luminescence from the two crystal phases resembles that of up-converted luminescence observed from the same host matrix following NIR excitation (i.e., hexagonal crystal emits stronger than the cubic crystal). However, down-conversion photoluminescence of nanoparticles under near UV excitation (365 nm) showed no appreciable difference in the luminescence intensities from two different phases (Figure 1d). A comparison to the X-ray excited luminescence shows that the crystal symmetry surrounding the Eu³⁺ ion under X-ray excitation was predominantly from a low symmetry site. The ⁵D₀→⁷F₁/⁵D₀→⁷F₂ ratio indicates that the local crystal structure surrounding the doped Eu³⁺ ions remains the same in the cubic and the hexagonal NaGdF₄ phases. Since the UV-excited luminescence intensity for ⁵D₀→⁷F₂ and ⁵D₀→⁷F₁ remains the same for both structures, albeit smaller at 1.3, it can be deduced that the degree of randomness associated with cations (Na⁺, Gd³⁺, and Eu³⁺) was similar within these crystal structures. The order–disorder of the cations in AlF₃ structures are known to be associated with the kinetics of heating. Since the hexagonal NaGdF₄:Eu³⁺ NPs were synthesized by a rapid hydrothermal method, a high degree of randomness in the cation distribution is to be expected.

In order to further understand the effect of crystal structure and compare the luminescence efficiencies, we compared the emission intensities of NaGdF₄:Eu³⁺ with other types of X-ray luminescent NPs reported in the literature (i.e., BaYF₅:Eu, Gd₂O₃:Eu, and BaF₂:Eu). Two phosphor systems were synthesized by a rapid hydrothermal method (Figure S1 Supporting Information). A high temperature monoclinic phase of Gd₂O₃:18%Eu³⁺ was synthesized with a flame spray pyrolysis developed by our group. The luminescence intensities of BaYF₅:Eu³⁺ were lower by more than a factor of 2 when compared with hexagonal NaGdF₄:Eu³⁺ (Figure 2). There was no significant improvement in the luminescence from BaYF₅:10%Eu³⁺ upon doping with 5–20% Ce³⁺ sensitizer ions. In addition, flame-synthesized Gd₂O₃:18%Eu³⁺ NPs that showed the brightest photoluminescence under near UV excitation also had low X-ray luminescence (Figure 2). Therefore, the results suggest that the luminescence obtained with near UV excitation may not be a good guide to the efficiency of luminescence that arises from X-ray excitation.

The X-ray luminescence from Eu³⁺ doped into hexagonal NaGdF₄ NPs was the brightest among the NPs that we studied. Our measurements indicated that the cubic crystal structure resulted in lower X-ray luminescence intensities in comparison to hexagonal structure, regardless of the host matrix and synthesis protocol. When cubic NaGdF₄:15%Eu³⁺ was compared with BaYF₅:10%Eu³⁺ and BaYF₅:10%Eu³⁺:5%Ce, the X-ray luminescence intensity of the NaGdF₄:15%Eu³⁺ was the highest. In contrast to NaGdF₄:15%Eu³⁺ nanoparticles, X-ray luminescence from BaYF₅:10%Eu³⁺ and BaYF₅:10%Eu³⁺:5%Ce appeared to be from Eu³⁺ in a more symmetric environment, as the ratio of ⁵D₀→⁷F₁/⁵D₀→⁷F₂ intensities were <1 (0.8), and a higher intensity of the ⁵D₀→⁷F₁ transition was observed.

X-ray luminescence from phosphors involves formation of (a) excitons (electron–hole pairs); (b) their thermalization; (c) transition of exciton energy to the luminescent center; (d) photon emission following the relaxation of the excited electron on the luminescent center; (e) heat generation. The X-ray excitation leads to the formation of electron–hole pairs with energy greater than or equal to the band gap of the material. The relaxation process proceeds either through a radiative pathway or by heat dissipation. X-ray excited luminescence is influenced by the activator ion, interstitial fluorides ions, and the dynamics of the electron–hole pairs in the presence of such impurity centers. The UV excited luminescence of cubic and hexagonal NaGdF₄:Eu³⁺ suggests that the efficiencies of the phosphors are similar. If the phonon density of state was an important factor that affected the emission, higher emission intensities from hexagonal nanoparticles would be expected.

It has been suggested that Ln³⁺ ions provide an effective mechanism for radiative emission by excitons generated by X-ray irradiation in a fluoride matrix. To constitute an efficient pathway of emission through rare-earth ions, these excitons must be stable and STE emission should be absent. The presence of Ln³⁺ ions in a fluoride matrix fulfills these two criteria. For AlN₄ structures, cross-luminescence from the valence band to the core band is not a major radiative pathway; the results suggest that visible luminescence is possible through migration of excitons to the doped ion. Therefore, the radiative decay of the conduction band electron through the activator ion in NaGdF₄ can be expected. The direct relaxation of the STE from the conduction band to the valence band is suppressed by the broadening of the valence band that is formed by the 2p electrons of the fluorine, transferring the holes to the Eu³⁺ ions that trap the excitons sequentially. This is followed by a radiative f→f transition that is promoted by the
broadening of the valence band in hexagonal NaGdF₄—
broadening of the valence band is expected in hexagonal
NaGdF₄ in comparison to cubic NaGdF₄ as there are two
different sites of fluorine in the unit-cell. It must be noted that
the cubic NaGdF₄ has eight coordinated holes that trap
electrons and contribute to nonradiative quenching through
reduced migration of the exciton energy to the Eu³⁺ ion. In the
case of BaYF₅ nanoparticles, the nonradiative quenching of the
excitons by LnFₓ in BaFₓ solids is well-documented.

Because X-ray luminescence was sensitive to the impurity
state, the nature of the nanoparticle surface can also be
expected to influence the X-ray luminescence. The pH of the
suspending medium can influence surface states on particles
and can possibly influence the X-ray luminescence. It is well-
known from the quantum dot literature that the local
environment affects the exciton emission and can be controlled
by introducing quenching centers such as thiols that diminish
emission. The NaGdF₄ particles studied in this work were
prepared by a citrate method giving the particles a surface
coating of citrate ions. Protonation/deprotonation of the
surface groups, in particular carboxylic acids on the citric acid that occurs between pH 2
− 7 with pKₐ values of 3.13, 4.76, and 6.4 changes the nature of the
surface groups, influencing surface defects. We observed that the X-ray luminescence of hexagonal NaGdF₄:15%Eu³⁺
decreased with a corresponding decrease in the pH of the solution (Figure 3) with the intensities of the ⁷D₀−⁷F₅
transitions of Eu³⁺ showing particular sensitivity to the pH. The modulation of the X-ray luminescence with changing pH was consistent—regardless of the particle concentration (see Supporting Information Figure S2). Furthermore, the ⁷D₀−⁷F₅/⁷D₀−⁷F₁ ratio remained unchanged (approximately 1.6−1.7) for all the pH values that were studied. On the other hand, UV-excited photoluminescence (excitation at 254 and at 365 nm) did not show a significant change due to variation of the pH. Hence, in combination, the UV and X-ray data rule out the possibility that the effect could be due to the crystal field changes around the Eu³⁺ ion. The UV-excited photoluminescence results suggest that the excited 4f electrons within the lanthanide are less sensitive to the environment. Thus, the results strongly suggest that the excitons generated by X-ray excitation are highly sensitive to adsorbed surface groups, defects, and defect-like features (such as broadening of the valence band) in NaGdF₄ nanoparticles. The pH sensitivity of the emission following X-ray excitation could, in principle, be used as an indicator of the local intracellular environment surrounding the particles, for example, distinguishing between the contrast agent that is present in lysosomes and cytoplasm.

Recently, Chen et al. demonstrated the monitoring of pH through X-ray luminescence based on pH-induced changes in the concentration of a material that has its optical absorption overlapping the luminescence of Gd₂O₃:S:Tb—shell nanoparticles. In this case, the optically active material was released in a controlled manner following changes in the pH of the solution, increasing the X-ray luminescence. On the other hand, our results showed that the pH of the solution could directly affect the X-ray excited luminescence (and not the UV excited luminescence), presumably through alteration of the electron−hole stability within the nanoparticle.

Gold nanoparticles offer high contrast in X-ray tomography. Gold nanoparticles conjugated to near-infrared fluorescent dyes have been shown to be excellent markers for dual-mode imaging that combines X-ray computed tomography and fluorescence. Metals may introduce plasmonic effects. It is known that the emission from lanthanides is affected not just by the lanthanide ion crystal symmetry or size, but also by plasmonic perturbations that can be produced by metals such as silver and gold. In addition to possible plasmonic enhancement of emission, gold can also provide desirable biocompatibility. Given the high density of gold (19.3 g/cm³), we hypothesized that the use of an inorganic scintillator core with a metal shell to increase the stopping power of X-rays, in combination with the possible plasmonic enhancement and biocompatibility, should make a phosphor with a gold shell a good candidate for in vivo imaging. However, little is known about the impact of gold on the X-ray luminescence from lanthanide ions. Previously, we have demonstrated a facile one-pot technique to coat gold on NaLnF₄ nanoparticles. In this work, we employed the same protocol to coat the gold on NaGdF₄ nanoparticles. Figure 4 shows a sample transmission electron microscopy image of the as-prepared gold-coated nanoparticles. In order to understand the effect of gold coating on the X-ray excited luminescence, various doping concentrations of Eu³⁺ in both hexagonal and cubic NaGdF₄ nanoparticles that were coated with gold were studied. The concentration of the particles in suspension was held constant in all cases to within a very small tolerance.

![Figure 3. Effect of pH on luminescence of hexagonal NaGdF₄:15% Eu³⁺ nanoparticles. (a) Under X-ray excitation and (b) UV-excitation (365 nm excitation).](image)

![Figure 4. (a) TEM micrographs of as-synthesized NaGdF₄:15% Eu³⁺@ Au. (b) X-ray excited luminescence of gold coated hexagonal and cubic NaGdF₄:Eu³⁺ for different Eu concentrations (3 mg/mL). (c) Comparison of X-ray excited luminescence spectra of gold-coated and uncoated hexagonal NaGdF₄:15% Eu³⁺ (d) Absorbance spectrum of gold-coated and uncoated hexagonal NaGdF₄:15% Eu³⁺ (1.5 mg/mL). Scale bars correspond to 100 nm.](image)
The cubic NaGdF₄:Eu³⁺ did not exhibit any significant dependence on the concentration of Eu³⁺ in the gold-coated particles (Figure 4b). For the hexagonal NaGdF₄:Eu³⁺, the dependence of X-ray luminescence on Eu³⁺ concentration was similar to that of uncoated nanoparticles. A Eu³⁺ doping concentration of 15% showed the highest luminescent intensity. However, in contrast to the up-converting nanoparticles, where it is known that a gold shell can enhance the up-converted luminescence, the ~5–8 nm thick gold shell (Figure 4a) in this case was found to reduce the X-ray excited luminescence. The total luminescence from NaGdF₄:15%Eu³⁺@Au was 75% of the uncoated material, with ^7D_0→^7F_1 and ^5D_0→^7F_2 transitions being 51% and 73% of the uncoated nanoparticle. Surprisingly, the ^7D_0→^7F_4 emission at around 700 nm wavelength in NaGdF₄:15%Eu³⁺@Au was fortuitously unaffected (given its relatively low absorbance in tissues) (Figure 4c).

The trend in the X-ray luminescence data can be explained by the taking into consideration the plasmonic absorption of the gold. Figure 4d shows the absorbance spectra of hexagonal NaGdF₄:15%Eu³⁺ and NaGdF₄:15%Eu³⁺@Au. The gold shell had a plasmonic peak around 530 nm. The plasmonic absorption decreased in the 550–700 nm region with increasing wavelength and the observed luminescence intensities from NaGdF₄:15%Eu³⁺@Au correlated with the plasmonic reabsorption. It should be noted that the experiments were performed in a cuvette with a defined width of 1 cm. It is therefore expected that the plasmonic reabsorption occurred not only from the gold coated on a given nanoparticle but also from the surrounding gold-coated nanoparticles as an inner filter effect.

To further understand the possible applicability of gold-coated and uncoated NaGdF₄:Eu³⁺ nanoparticles for in vitro imaging with human cells, cell viability studies were conducted. Primary human aortic endothelial cells were chosen as typical of endothelial cells that might be exposed to nanoparticles upon injection, as they line the inside of the blood vessels. Calcein AM is a nonfluorescent membrane permeant dye that is hydrolyzed by intracellular acetoxymethyl ester to a fluorescent Calcein conjugate, which then accumulates inside the cell and can be used to indicate membrane integrity and cellular viability (false colored green). Propidium iodide is a membrane impermeant nucleic acid stain that binds the DNA of dead cells and is normally excluded from viable cells (false colored red). A 20 h in vitro comparison of NaGdF₄:15%Eu³⁺ and NaGdF₄:15%Eu³⁺@Au showed that at the 50 μg/mL concentration there were no visible toxicity or viability changes associated with nanoparticle exposure to the cells, as can be seen by the minimal increase in propidium iodide signal and almost constant cell surface coverage observable through fluorescent Calcein (See Supporting Information Figure S3). At 250 μg/mL, it was found that the viability decreased for both gold-coated and uncoated particles (Figure 5a)—the gold-coated particles elicited less response (Figure 5b). Image J analysis was performed on previously shown Calcein images, in which total cell count and % surface area coverage were used as a means of quantifying cellular viability (See Supporting Information Figure S4). Using either cell count or % surface area, an analysis showed that gold-coated particles had a slightly less toxic effect on the cells compared to their corresponding noncapped particles. Human aortic endothelial cells exposed to 250 μg/mL NaGdF₄:15%Eu³⁺ had 34 viable cells covering 14.9% of the surface area (a) and NaGdF₄:15%Eu³⁺@Au had 41 viable cells covering 16.8% of the surface area (b) and the Calcein control had 49 viable cells covering 26.6% of the surface area (c). While the Calcein signal decreased, the propidium iodide signal did not increase proportionally, due to the inherently adherent nature of the cells. Following death, the cells ball-up and eventually detach from the surface, becoming freely suspended in the medium and thus out of the plane of imaging. As a result, the propidium iodide stain is not as effective a measure of viability as Calcein; we drew our conclusions from the Calcein images. It is not presumed that this assay is conclusive with regard to the relative cytotoxicity of bare vs gold-coated particles. However, for the purpose of this study, this experiment serves as a proof of concept that the addition of a gold coating has the potential to decrease cytotoxicity. A more in-depth cytotoxicity study will include additional types of cytotoxicity assays such as MITT, LDH, and MTS.

The suitability of NaGdF₄:Eu³⁺ particles in X-ray luminescence optical tomography was examined using a mouse model (Figure 6). The luminescence from the NaGdF₄:15% Eu³⁺ nanoparticles was bright in comparison to the BaYF₄:10%Eu³⁺ that has been used in the past for in vivo X-ray luminescence. The gold-coated phosphor also showed excellent brightness due to reduced interparticle absorption. Our results show that hexagonal NaGdF₄:Eu³⁺ nanoparticles are excellent candidates for applications that utilize X-ray activated luminescence and could have a future role for in vivo molecular imaging studies.

CONCLUSIONS

X-ray excited luminescence and photoluminescence of hexagonal and cubic NaGdF₄:Eu³⁺ nanophosphors in aqueous medium were studied. X-ray excited luminescence from the hexagonal phase was twice as strong as that from the cubic phase. Surprisingly, the emission intensity was found to be pH...
dependent. The UV-excited photoluminescence intensities, on the other hand, from these two phases were identical, indicating that the radiative emission of electron–hole pairs generated by X-ray excitation is influenced by the crystal structure and the local environment. Coating of the particles with a gold shell affected the overall emission of light but the most important NIR line near 700 nm was fortunately not affected, preserving the usefulness of the gold-coated particles for XLOT while improving their biocompatibility. Hexagonal NaGdF₄:Eu³⁺ nanoparticles with an optimal doping concentration of 15% Eu³⁺ are a suitable choice for X-ray luminescence optical imaging.

ASSOCIATED CONTENT
1 Supporting Information
Additional figures as mentioned in the text, characterization (X-ray, DLS, TEM), luminescence, absorbance spectra and schematic of experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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