NaGdF₄ Nanoparticle-Based Molecular Probes for Magnetic Resonance Imaging of Intraperitoneal Tumor Xenografts in Vivo

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ABSTRACT Differently sized NaGdF₄ nanocrystals with narrow particle size distributions were synthesized by a high temperature approach. Upon ligand exchange, the as-prepared hydrophobic NaGdF₄ nanocrystals were transferred into water by using asymmetric PEGs simultaneously bearing phosphate and maleimide groups. Further investigations demonstrated that the water-soluble NaGdF₄ nanocrystals, coated by PEG bearing two phosphate groups on the same side, exhibit not only excellent colloidal stability in water and PBS buffer, but also higher T₁ relaxivity than Gd-DTPA (Magnevist). Through "click" reaction between the maleimide residue on particle surface and thiol group from the partly reduced anti-EGFR monoclonal antibody (mAb), NaGdF₄—PEG—mAb nanoprobes were constructed, and their biocompatibility and binding specificity were evaluated through in vitro experiments. A series of in vivo experiments were then carried out for detecting intraperitoneal tumor xenografts in nude mice by using magnetic resonance (MR) imaging technique. The results revealed that the NaGdF₄—PEG—mAb probes possessed satisfying tumor-specific targeting ability and strong MR contrast enhancement effects.

KEYWORDS: NaGdF₄ nanoparticles, ligand exchange, T₁ contrast agent, MR molecular imaging probe, intraperitoneal tumor xenografts

Magnetic resonance imaging (MRI) has become one of the most important clinical tools nowadays for visualizing the internal structures of the body in detail. Despite extremely high spatial and temporal resolutions, MRI still suffers from its poor ability in differentiating diseased and healthy tissues. Therefore, developing MRI contrast agents is highly demanded.

To date, paramagnetic Gd-chelates dominate the MRI contrast agents used in clinical diagnosis, meanwhile the nanoparticle-based MRI contrast agents have received increasing attention since FDA approved two magnetic iron oxide particle-based contrast agents in 1996. Due to the enhanced vascular permeability, nanoparticles tend to be localized in tumor and therefore exhibit unique tumor-specific targeting ability in contrast to the paramagnetic metal chelates.

In addition, nanoparticle also offers a platform on which different types of functional moieties can be assembled for achieving multifunctional probes potentially useful for both diagnostic and therapeutic purposes. So far, contrast agents based on superparamagnetic iron oxide particles have widely been investigated and evaluated in clinical trials of different stages. In contrast, gadolinium-based paramagnetic nanoparticles as contrast agents are much less reported.

In literature, the gadolinium-based paramagnetic nanocrystals are typically synthesized through the replacement reactions taking place between Gd³⁺ and anions such as F⁻, PO₄³⁻, and OH⁻, respectively, in water and polyol, or the thermal decomposition reactions taking place in high boiling point organic solvents using different types of gadolinium-organic compounds as precursors. The first approach is

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straightforward for producing hydrophilic nanocrystals such as GdF$_3$, GdPO$_4$, and Gd$_2$O$_3$, etc., but the resultant nanocrystals are typically characterized by broad size distributions. In contrast, the second approach can give rise to nanocrystals with greatly improved size and shape uniformities. But it is only suitable for producing hydrophobic nanocrystals.

Aqueous solubility/dispersibility and colloidal stability are very important prerequisites for in vivo applications of any types of inorganic nanoparticles. Since the biodistribution, clearance rate, and elimination pathway of the intravenously injected nanoparticle are strongly associated with the particle size, an effective size control over the monodispersed Gd$_3$O$_2$-containing nanocrystals and a suitable postpreparative approach for endowing the hydrophobic nanocrystals with aqueous colloidal stability are therefore equally important. Different methods for rendering the oleate-coated nanoparticles water-soluble have been reported recently. Among them, overcoating the hydrophobic nanoparticles with SiO$_2$ has become the most popular one. But the precise control over the thickness and shape of SiO$_2$ encapsulating layer remains challenging. Other measures such as oxidizing the oleate ligand with ozone or potassium permanganate, or replacing the oleate ligand with polyethylene glycol (PEG) or polivinlypyrrolidone were also explored. But NaGdF$_4$ nanoparticles with good colloidal stability under physiological condition are rarely reported so far.

Herein, we report the preparation of nearly monodispersed NaGdF$_4$ nanocrystals, following a synthetic approach initially reported by Zhang. Preparation was optimized so as to get differently sized NaGdF$_4$ nanocrystals stabilized by oleic acid. Upon ligand exchange, water-soluble NaGdF$_4$ particles bearing surface reactive maleimide (mal) groups were obtained. Through efficient “click” reaction, tumor-specific MRI probes were constructed, evaluated, and subsequently used in detecting intraperitoneal tumor xenografts in nude mice.

RESULTS AND DISCUSSION

Synthesis and Characterization of NaGdF$_4$ Nanoparticles. In brief, NaGdF$_4$ nanocrystals were prepared by a high temperature approach through the replacement reaction between GdCl$_3$ and NH$_4$F taking place in the presence of excess NaOH. Oleic acid (OA) was chosen as a particle surface capping agent. It also served as solvent together with 1-octadecene. The particle size and size distribution shown in Figure S1 in Supporting Information (SI) reveal that the NaGdF$_4$ particle growth undergoes a size broadening process at 300 °C, followed by a size focusing process, suggesting that the Ostwald ripening process plays an important role determining both particle size and size distribution in the current reaction system. Therefore, the reaction temperature and time were optimized so as to vary the size of NaGdF$_4$ nanoparticles and meanwhile narrow their size distribution.

The transmission electron microscopy (TEM) images shown in Figure 1 demonstrate that the size of monodispersed particles obtained can effectively be tuned by different combinations of the reaction time and temperature, for example, 45 min at 270 °C to achieve 5.4 ± 0.8 nm NaGdF$_4$ (NaGdF$_4$-5), 60 min at 300 °C to get 15.1 ± 0.8 nm NaGdF$_4$ (NaGdF$_4$-15), and 60 min at 320 °C to obtain 19.8 ± 1.0 nm NaGdF$_4$ (NaGdF$_4$-20), respectively, which marks the difference of the current synthetic route from previous ones by its strong particle size tunability. Further powder X-ray diffraction (XRD) studies reveal that NaGdF$_4$-5 is in the cubic-phase, while NaGdF$_4$-15 and NaGdF$_4$-20 are in the hexagonal-phase.

Preparation and Characterization of Water-Soluble NaGdF$_4$ Nanoparticles. To render the NaGdF$_4$ nanocrystals stabilized by oleic acid water-soluble, asymmetric polyethylene glycols (PEGs) carrying a maleimide group at one end and one or two phosphate groups at the other end, denoted as mal-PEG-m and mal-PEG-dp, respectively, were used to replace the oleate ligand of the as-prepared NaGdF$_4$ nanocrystals based on the fact that the phosphate group has a higher binding affinity to Gd$^{3+}$ than the carboxyl group from oleic acid. The detail chemical structures of mal-PEG-m and mal-PEG-dp are shown in Scheme 1. Dynamic light scattering (DLS) analysis was carried out to characterize the aqueous dispersion of the resultant nanoparticles. As shown in Figure 2a, NaGdF$_4$-20 particles stabilized by mal-PEG-m exhibit a relatively narrow particle size distribution in water with a single scattering peak locating at 51 nm. The DLS result on the one hand suggests that PEG-phosphate can effectively replace oleate ligand, on the other hand indicates that the ligand exchange process took place in a controlled way that no unwanted agglomeration of the particles occurred.

Satisfying colloidal stability is a very important prerequisite for exploring the biomedical applications of nanomaterials since this stability strongly governs the blood circulation behavior of the nanoparticles administrated via intravenous injection and the resultant particle biodistribution as well. Therefore, the colloidal stability of (mal-PEG-m)-coated NaGdF$_4$ particles in water and phosphate buffered saline (PBS) that is commonly used to mimic the human biological conditions was evaluated by DLS method. As shown in Figure 2b, (mal-PEG-m)-coated NaGdF$_4$-20 particles present excellent colloidal stability in pure water. However, they quickly flocculate in 1× PBS as illustrated by the drastic increase in the hydrodynamic size of the particle aggregates. The quick flocculation is mainly caused by the replacement of mal-PEG-m ligand by phosphate ion in PBS as the latter is very
excessive in the system. To solve this problem, mal-PEG-dp was adopted instead of mal-PEG-mp to exchange the oleate ligand of the NaGdF4 nanoparticles so as to enhance the colloidal stability of the resultant nanoparticles in phosphate buffer solution. The temporal evolutions of the hydrodynamic size of (mal-PEG-dp)-coated NaGdF4-20 particles in both water and PBS, as shown in Figure 2c, strongly support that mal-PEG-dp ligand can more effectively prevent NaGdF4 nanocrystals from forming aggregates under physiological conditions. The greatly improved colloidal stability can be interpreted by the multidentate Gd$^{3+}$ binding ability of the diphosphate group. Further experimental observation demonstrated that the (mal-PEG-dp)-coated NaGdF4 nanocrystals remained colloidal stable in PBS since they were prepared more than 1 year ago.

**Relaxivity Measurement in Vitro and Cytotoxicity of PEG-Coated NaGdF4 Nanoparticles.** The performance of the (mal-PEG-dp)-coated NaGdF4 nanoparticles as MRI contrast agents was evaluated on a clinic 3T MRI scanner. The experimentally determined longitudinal relaxivity $R_1$ of water protons was plotted against the molar concentration of Gd$^{3+}$. The molar relaxivity $r_1$ extracted from the linear regression fits of the experimental data, as shown in Figure 3a, is of 6.20 mM$^{-1}$ s$^{-1}$ for NaGdF4-5, 5.7 mM$^{-1}$ s$^{-1}$ for NaGdF4-15, 8.78 mM$^{-1}$ s$^{-1}$ for NaGdF4-20, and 3.15 mM$^{-1}$ s$^{-1}$ for Gd-DTPA (DTPA = diethylenetriaminepentaacetate). In comparison with Gd-DTPA, the first paramagnetic contrast agent marketed as Magnevist for clinical use, the NaGdF4 nanoparticles present greatly enhanced $r_1$ that shows a nonmonotonic behavior against the particle size. This can qualitatively be interpreted by the following particle size-related effects. On the one hand, $r_1$ relaxivity should decrease with the NaGdF4 particle size since the amount of surface Gd$^{3+}$, the main driving force for shortening the longitudinal relaxation of water protons nearby, decreases against the particle size at a given concentration of Gd$^{3+}$. On the other hand, this effect may be counteracted by the decreased tumbling time for larger particles. In fact, according to the impact of a contrast agent on the relaxation of water protons, the surrounding water can be divided into three distinct regions: inner sphere, secondary sphere, and outer

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Figure 1. TEM images and histograms of the as-prepared NaGdF4 nanoparticles: NaGdF4-5 (a), NaGdF4-15 (b), and NaGdF4-20 (c). The scale bars correspond to 50 nm.
sphere.24 The relaxation of magnetization of water protons in the secondary and outer spheres are mainly contributed by the water molecules bonded to the particle surface ligands and their exchange with bulk water.25 Therefore, it can be deduced that the water protons in the inner sphere contribute to the particle size-dependent r1 relaxivity since the surface ligands for these three particle samples are the same for the present study. The longitudinal relaxivity of water protons in the inner sphere is strongly dependent on the tumbling time of the contrast agent, that is, the NaGdF4 particle in the current case.18 In principle, slowing the tumbling of Gd3+ ions is in favor of higher r1 relaxivity, while the tumbling time decreases as the nanoparticle size increases. This may explain why NaGdF4-20 presents a higher r1 than NaGdF4-15. Although the aforementioned two particle size-dependent effects on r1 can help to qualitatively understand the particle size dependency of r1, more systematic investigations are still needed for further quantitatively interpreting the particle size-dependent r1. Nonetheless, the current results give strong enough reason to further study NaGdF4-5 and NaGdF4-20 as they both present enhanced relaxivity in comparison with NaGdF4-15 apart from a bigger mutual difference in size.

Before further in vitro and in vivo experiments, the cytotoxicity of the (mal-PEG-dp)-coated NaGdF4-5 and NaGdF4-20 was first evaluated through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assays on GC7901 cells. The results are shown in Figure 3b. In comparison with Gd-DTPA, both NaGdF4-5 and NaGdF4-20 give rise to enhanced cell viability when the concentration of Gd3+ is below 5 mM. However, the NaGdF4 nanoparticles start to decrease the cell viability when the concentration of Gd3+ is higher than 10 mM, more dramatically than Gd-DTPA. Theoretical fittings reveal that the IC50 (50% inhibitory concentration) values of NaGdF4-5 and NaGdF4-20 are of 16.5 and 13.8 mM, respectively. Since the highest clinical dose for Gd-DTPA is around 0.3 mmol·kg⁻¹, roughly corresponding to 0.3 mM, it is reasonable to

![Scheme 1. Molecular structures of mal-PEG-mp and mal-PEG-dp ligands.](image)

![Figure 2. Hydrodynamic size profile of the (mal-PEG-dp)-coated NaGdF4-20 nanoparticles (a), and temporal evolutions of the hydrodynamic sizes of the (mal-PEG-mp)-coated (b) and the (mal-PEG-dp)-coated NaGdF4-20 nanoparticles (c) in water and 1×PBS, respectively.](image)

![Figure 3. (a) R1 relaxivity of aqueous solutions containing differently sized nanoparticles or Gd-DTPA with different concentrations of Gd3++; (b) cell viability of GC7901 cells incubated with (mal-PEG-dp)-coated NaGdF4 particles or Gd-DTPA with different Gd3+ concentrations.](image)
believe that the current NaGdF₄-5 and NaGdF₄-20 are on the safe side for being used as contrast agent in MR imaging, at least for animal studies.

Conjugation of anti-EGFR mAb to NaGdF₄ Nanoparticles. Two tumor-specific MR molecular probes were prepared by covalently conjugating anti-EGFR monoclonal antibody (mAb) to the NaGdF₄-5 and NaGdF₄-20 through the “click” reaction between the maleimide residue on particle surface and thiol group from the partly reduced anti-EGFR mAb. The effectiveness of the coupling reaction was first evaluated by DLS. The results shown in Figure S2 in the SI reveal that the hydrodynamic size of NaGdF₄-20 increases from 51 to 57 nm after the coupling reaction and the size distribution profile of the conjugates remains nearly unchanged in comparison with that of the mother particles, which strongly supports that the mAb molecules are effectively coupled to the nanoparticles. Further spectroscopy studies demonstrated that the resultant conjugates were formed through the maleimide–thiol “click” reaction rather than the nonspecific adsorption of mAb on the particles (more details are provided in Figure S3 in SI).

Maintaining the bioactivity of anti-EGFR mAb is of the utmost importance for the following application of the resultant NaGdF₄–PEG-mAb conjugates. The bioactivity of the NaGdF₄–PEG-mAb conjugates was quantitatively evaluated through cell binding assays in which human epithelial carcinoma cell line A431 and human breast carcinoma cell line MDA-MB-435 were used as positive and negative controls, respectively. The difference in the binding affinity of the NaGdF₄–PEG-mAb probe to A431 and its control MDA-MB-435 can constructively be visualized from the fluorescence micrographs shown in the upper panel of Figure 4. The quantitative analysis through ICP–AES measurements revealed that the amount of NaGdF₄-20 in A431 cells was 10-fold of that in MDA-MB-435 after they were treated with the nanoprobes, suggesting that the NaGdF₄–PEG-mAb conjugates possess excellent binding specificity to tumor cells overexpressed with EGFR on the surface. In addition, murine immunoglobulin G (mIgG) was chosen to construct a control conjugate, that is, NaGdF₄–PEG-mIgG, for cell binding assays. The results shown in the lower panel of Figure 4 strongly support that the binding specificity of the NaGdF₄–PEG-mAb conjugate is endowed by the specific interaction between EGFR and anti-EGFR antibody, while the nonspecific uptake of the irrelevant NaGdF₄–PEG-mIgG probe is rather weak.

In Vivo MR Imaging of Intraperitoneal Tumor Xenografts. In previous investigations, the subcutaneously xenografted tumors were the most commonly used tumor model for molecular imaging studies using nanoparticle-based molecular probes. In the current study, the intraperitoneally xenografted tumor model was used instead for better reflecting the nature of colorectal cancer as the latter can also be taken as a metastatic model. On the basis of the aforementioned systematic characterizations as well as successful in vitro experiments, NaGdF₄–PEG-mAb probes were used in the following in vivo experiments for detecting colorectal tumors in BALB/c nude mice, and the corresponding mother particles and Gd-DTPA were used as negative controls. Since the NaGdF₄–PEG-mIgG probe did not present enhanced interactions with A431 cells in vitro in comparison with the corresponding mother particle, it was not further adopted as a control for further in vivo imaging experiments. T1-weighted MR images acquired before and at different time points postinjection are provided in the upper panel of Figure 5. It is quite evident that the NaGdF₄–PEG-mAb probes present clear tumor targeting ability in vivo. In contrast, Gd-DTPA hardly enhances the contrast of the tumor region under the same experimental conditions, as shown in Figure S4 in SI. The tumor-targeting behaviors of the NaGdF₄–PEG-mAb probes can clearly be seen from the quantitative analysis on the temporal evolutions of T1 values in the tumor regions, as shown in the lower frames in Figure 5. In general, the T1 values of the tumor site start to decrease shortly after the injection of NaGdF₄–PEG-mAb probes, reaching a minimum at ~4 h postinjection by AT1 of ~50% for NaGdF₄-5-mAb and 30% for NaGdF₄-20-mAb. Although the mother particles also present similar temporal evolutions of T1 values in the tumor regions, as shown in the lower frames in Figure 5.

Figure 4. (Upper panel) Fluorescence images of A431 cells (left) and MDA-MB-435 cells (right) stained by NaGdF₄–PEG-mAb probes and Hoechst 33342 for cell membrane and nucleus, respectively; (lower panel) Gadolinium concentrations in A431 and MDA-MB-435 cell samples determined after they were treated with NaGdF₄, NaGdF₄–PEG-mAb, and NaGdF₄–PEG-mIgG, respectively, while the gadolinium concentrations of untreated A431 cells and MDA-MB-435 samples are shown as “blank”.

Figure 5. T1-weighted MR images acquired before and at different time points postinjection in BALB/c nude mice of intraperitoneal xenograft tumors were obtained using (A) NaGdF₄–PEG-mAb; (B) NaGdF₄–PEG-mIgG; (C) Gd-DTPA; and (D) water as control. T1 values are represented as mean ± standard error of mean (n = 3).
behaviors, quite probably caused by the enhanced permeability and retention effect associated with tumor, the resulting \( \Delta T1 \) is much lower (<15%). It should be mentioned that the variation of the relative tumor position in the same mouse is mainly caused by the displacement of the internal organs and tumor induced by repeatedly fixing the mouse on a respiration sensor for monitoring the living status of the mice under anesthesia during the MRI experiments.

To provide further support to the tumor-specific targeting ability as well as information on the biodistribution of the NaGdF\(_4\)–PEG-mAb probes, both tumors and important organs such as liver, spleen, and kidney were harvested after the in vivo experiments. Then Gd contents in the tumor tissue and different organs were analyzed by ICP–AES. The results on tumor uptakes of the NaGdF\(_4\)–PEG-mAb probes and the mother particles, as shown in Figure 6, are generally well in consistency with the imaging results shown in Figure 5. In comparison with similarly structured tumor probes based on Fe\(_3\)O\(_4\) particles,\(^8\) the current NaGdF\(_4\)–PEG-mAb probes present greatly enhanced tumor uptake efficiencies, by at least a factor of 2.4, if the corresponding liver uptake is used as internal reference representing the mononuclear phagocyte system. Nevertheless, similar to most particulate probes, the liver uptakes of the probes and the control particles remain in spite of reduced levels. It should also be pointed out that the data shown in Figure 6, as follow-up results of the in vivo experiments, are far from complete for describing the pharmacokinetics of the new probes reported herein. In addition, nothing is known about the clearance rate and elimination pathways of current probes at present. But the current investigations have demonstrated that the NaGdF\(_4\) nanocrystals as T1 contrast agents are potentially useful for constructing MRI probes for in vivo tumor imaging, as an alternative choice of T2 contrast agents formed by iron oxide nanoparticles, at least for animal studies.

**CONCLUSIONS**

In summary, the asymmetric PEGs simultaneously bearing phosphate and maleimide groups were designed and used to exchange the oleate ligand of NaGdF\(_4\) nanocrystals, on the one hand for rendering them water-soluble and on the other hand for further covalently conjugating tumor-specific ligand to the nanoparticle to form molecular probe for MR imaging.
of tumors. Systematic studies demonstrated that PEG-phosphate can effectively replace the oleate ligand, but the PEG bearing single phosphate group cannot firmly anchor on the particle surface in PBS. In contrast, the PEG ligand carrying two phosphate groups on the same side presents greatly improved binding affinity to the NaGdF₄ nanoparticles. Consequently, the NaGdF₄ nanocrystals exhibit long-term colloidal stability in both water and PBS buffer. In addition, the water-soluble NaGdF₄ nanoparticles also present superior contrast agent properties to Gd-DTPA.

**EXPERIMENTAL SECTION**

**Chemicals.** The following materials were purchased from Sigma-Aldrich: GdCl₃·6H₂O, oleic acid (OA), 1-octadecene (ODE), ammonium fluoride (NH₄F), tri(2-carboxyethyl)phosphine hydrochloride (TCEP), and methyl thiazolyl tetrazolium (MTT). Analytical grade chemicals such as ethanol, cyclohexane, and tetrahydrofuran (THF) were purchased from Sinopharm Chemical Reagent Beijing, Co., Ltd. PEGs (M₉₀ ≈ 2000) with one or two phosphate groups at one end of the chain and a maleimide group at the other end were custom products provided by Beijing Oneder Hightech Co. Ltd. Human gastric cancer cell line GC7901, human epithelial carcinoma cell line A431, human breast carcinoma cell line MDA-MB-435, and human colorectal cancer cell line LS180 were obtained from the Oncology School of Peking University. Monoclonal anti-EGFR antibody (Erbitux) was purchased from Boehringer Ingelheim Pharma GmbH & Co. KG. Murine immunoglobulin G (mIgG) was purchased from Boehringer Ingelheim Pharma GmbH & Co. KG. KG. Murine immunoglobulin G (mIgG) was purchased from Boehringer Ingelheim Pharma GmbH & Co. KG. Murine immunoglobulin G (mIgG) was purchased from Boehringer Ingelheim Pharma GmbH & Co. KG.

**Preparation of NaGdF₄ Nanoparticles.** In a typical preparation, GdCl₃·6H₂O (0.371 g, 1 mmol) was dissolved in a mixture of OA (14 mL) and ODE (16 mL). The solution was heated to 150 °C under nitrogen protection to form a homogenous solution, into which 10 mL of methanol solution containing NaOH (0.100 g, 2.5 mmol) and NH₄F (0.148 g, 4 mmol) was then slowly added after it was cooled down to room temperature. After that, the reaction mixture was kept under stirring at 50 °C for 30 min, at 100 °C under vacuum for 10 min to remove methanol, and then heated to 300 °C under atmospheric pressure by electromantle, and maintained at 300 °C for 1 h under nitrogen protection. The preparation was terminated by cooling the reaction mixture down to room temperature. The resultant nanoparticles, that is, NaGdF₄·15, were precipitated by ethanol, collected by centrifugation, washed with ethanol several times, and finally dispersed in THF or cyclohexane for further experiments.

**Ligand Exchange.** As a typical example, 100 mg of PEG-phosphate ligand was mixed with ca. 10 mg OA-coated NaGdF₄ nanoparticles in 10 mL of THF. Then, the reaction mixture was kept overnight at room temperature under stirring. The PEG-coated particles were precipitated by cyclohexane, washed with cyclohexane for three times, and finally dried under vacuum at room temperature. The PEG-coated NaGdF₄ particles obtained in this way, independent of the particle size, were found to be readily dissolved in aqueous media.

**Relaxivity Measurements.** The relaxivity measurements were carried out on a 3 T clinical MRI instrument (GE signa 3.0T HD, Milwaukee, WI). A series of aqueous solutions of (mal-PEG-dp)-coated NaGdF₄ particles in 1.5 mL Eppendorf tubes were prepared. The parameters for T₁ measurements were set as follows: echo time (TE) = 15.3 ms; repetition time (TR) = 500, 1000, 1500, 2000 ms; number of excitations (NEX) = 8.

**Cytotoxicity of PEG-Coated NaGdF₄ Nanoparticles.** MTT assays on GC7901 cells were carried out as follows. Cells were seeded into a 96-well cell culture plate by 2 × 10³ cells/well under 100% humidity, and then cultured at 37 °C in an atmosphere containing 5% CO₂ for 24 h. The (mal-PEG-dp)-coated NaGdF₄ nanoparticles were added to the wells at designed concentrations and incubated with the cells for 24 h at 37 °C under 5% CO₂. Subsequently, the supernatant containing the (mal-PEG-dp)-coated NaGdF₄ nanoparticles was decanted, and the cells were incubated for another 48 h at 37 °C under 5% CO₂. Thereafter, MTT (10 μL, 5 mg/mL) was added to each well and incubated for 4 h at 37 °C under 5% CO₂. After addition of 100 μL of DMSO per well, the assay plate was shaken at 37 °C for 10 min. The optical density of each well at 570 nm was recorded on a microplate reader (Thermo, Varioskan Flash), while the optical density at 690 nm was used as reference.

**Preparation of NaGdF₄—PEG-mAb Conjugates.** Typically, anti-EGFR mAb (1 mg/mL in 10 mL of PBS) was subjected to mild reduction by TCEP to convert the disulfide groups in the Fc fragments to thiols. The reduction and subsequent purification was performed in 1.5 mL of 30 K MWCO centrifugal devices (Millipore YM-30). The following conjugation reaction was performed by mixing (mal-PEG-dp)-coated NaGdF₄ nanoparticles with the partially reduced anti-EGFR mAb in Tris-buffered saline (TBS, pH 7.4). In principle, the yield of the "click" reaction between maleimide and thiol groups is close to 100%. In the current imaging probe preparation, the concentration of antibody was much smaller than that of the maleimide residue from the particle surface. Nevertheless, the NaGdF₄—PEG-mAb conjugates were purified by centrifugation for 3 times at 16000g to remove the unreacted antibody, and then stored at 4 °C. FITC-labeled anti-EGFR mAb was also used to prepare fluorescent particle probes following the above procedures.

**Binding Specificity of NaGdF₄—PEG-mAb Conjugates.** Fluorescence microscopy was used for qualitative evaluation of the binding specificity of the NaGdF₄—PEG-mAb probes to A431 and MDA-MB-435 cells. In detail, approximately 1 × 10⁵ A431 and MDA-MB-435 cells were seeded in the wells of two 8-well chamber slides, respectively, and incubated overnight at 37 °C under 5% CO₂ to allow a firm adherence. After being rinsed with PBS buffer, the cells were incubated with NaGdF₄—PEG-mAb conjugates labeled by FITC at 37 °C under 5% CO₂ for 1 h. After that, the cells were rinsed three times with PBS buffer. The fluorescence micrographs were captured using a fluorescence microscope (Olympus X71). The cell-associated Gd³⁺ was determined by using inductively coupled plasma-atomic emission spectrometer (ICP-AES) after the cells were eroded by 3 M HNO₃.

**Animal Tumor Model.** The tumor model used was established upon intraperitoneal injection of ~5 × 10⁵ LS180 cells into 4–6-week-old male BALB/c nude mice. The tumor imaging studies were carried out when the tumor volume reached 180–240 mm³ (~10 days after inoculation). All the experiments were performed according to a protocol approved by the Peking University Institutional Animal Care and Use Committee.
In Vivo MR Imaging of Intrapertoneal Tumor Xenografts. Nude mice bearing LS180 tumors in the enterocoelia regions were anesthetized, and then via tail vein the NaGdF4–PEG–mAb probe, the mother particle, or Gd-DTPA was injected by 0.1 mmol Gd(III)/kg body weight. MR imaging was conducted on a 4.7 T/30 cm Bruker Biospec animal MRI instrument using a saturation-recovery spin–echo imaging sequence. The imaging parameters were set as follows: field of view (FOV) = 3.5 × 4.5 cm²; matrix size = 128 × 128; slice thickness = 1 mm; echo time (TE) = 11 ms; repetition time (TR) = 90, 150, 300, 500, 800, 1200, 2000, and 3000 ms; number of excitations (NEX) = 4. T1 maps were calculated by pixel-wise fitting of the TR-dependent signal intensity changes to a single exponential function. The mice were eroded by HNO₃ and H₂O₂.

Characterizations. TEM images of the nanocrystals were taken on a JEM-100CXII electron microscope at an acceleration voltage of 100 kV. The particle size was determined by counting at least 300 nanocrystals per sample. Powder XRD patterns of the particle samples were recorded on a Regaku D/MAX-2500 diffractometer under Cu Kα radiation (λ = 1.54056 Å). DLS measurements were carried out at 298.0 K with Nano ZS (Malvern) equipped with a solid state He–Ne laser (λ = 632.8 nm) for determining the hydrodynamic size of the PEG-coated NaGdF₄ and monitoring the following bioconjugation reaction. The concentration of Gd(III) was determined by using an inductively coupled plasma atomic emission spectrometer (ICP-2000) produced by Jiangsu Skyray Instrument Co., Ltd.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Additional figures as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES