An efficient dye-sensitized NIR emissive lanthanide nanomaterial and its application in fluorescence-guided peritumoral lymph node dissection†

Qingyun Liu, Xianmei Zou, Yibing Shi, Bin Shen, Cong Cao, Shengming Cheng, Wei Feng id, e and Fuyou Li id, e

The luminescence intensity of near-infrared (NIR) emitting lanthanide nanoparticles (LnNPs) is usually limited, owing to their small absorption cross section. Although dye sensitization has been proven to be an effective way to improve the luminescence intensity of LnNPs, the sensitization effect is fairly limited, owing to the simplicity of the sensitizers used and the complexity of the energy transfer process, typically involving three steps. In this study, a more efficient sensitizer (Cy7) was chosen to replace a commonly used one (ICG) and the energy transfer process was also optimized through using Yb3+ ions as emitter ions and Nd3+ ions as intermediate ions. With Cy7 as a sensitizer, the sensitization effect was assessed to be better than with ICG, owing to the higher quantum yield of Cy7. Meanwhile, the Cy7-sensitized NIR lanthanide nanomaterial was proven to be good for deep tissue penetration and low-power excitation bioimaging. Furthermore, the highly-enhanced NIR signal was successfully used in blood vessel imaging and fluorescence-guided peritumoral lymph node dissection in a mouse model.

1 Introduction

Optical imaging is a non-invasive and real time imaging method with high spatial resolution and sensitivity, making it very important in the detection, diagnosis and therapy of diseases.1–3 However, imaging in the visible region has been found to suffer from high autofluorescence background signals and low tissue penetration depth, owing to light attenuation in biological tissue, which significantly limit the development of optical imaging.4,5 In order to overcome the aforementioned problems, considerable efforts have been made in relation to the exploitation of near infrared (NIR) optical imaging. Near infrared light from 700–1100 nm is known as the “optical transparency window” and can penetrate deeper tissue than the visible light, because absorption and scattering from blood, skin and fatty tissue are quite low in this window.6–8 Moreover, there is almost an absence of tissue autofluorescence, resulting in imaging with a high signal-to-background ratio. Therefore, NIR to NIR bioimaging can help attain outstanding imaging results.9–11 To date, many NIR-emitting materials have been developed for bioimaging, such as organic dyes,12 quantum dots13 and carbon nanotubes.14 Compared with these fluorescent materials, lanthanide-doped nanoparticles (LnNPs) exhibit superior optical properties, such as high optical and chemical stability, low toxicity, narrow band emission and a long emission lifetime.15–17 These properties make lanthanide materials more suitable for bioimaging. However, the emission intensity of LnNPs is relatively weak, due to the characteristics of their f–f transitions.18 Generally, when LnNPs were used for bioapplications, owing to their low emission intensity, a high excitation power density was needed to acquire better imaging results, which may cause overheating effects and tissue damage.19 To improve the emission intensity of LnNPs, enlarging the absorption cross section is an effective method.20 Recently, many studies have focused on using organic dyes as sensitizers of lanthanide ions to enlarge the absorption cross section, thus improving the emission intensity, and have got satisfying results.21,22 For example, Hummelen’s group used organic infrared dyes as sensitizers, enabling the broadband excitation of NaYF4: Yb/Er.22 Subsequently, Prasad et al. introduced the concept of an energy-cascaded upconversion system, in which they utilized the designing of NIR dye sensitized core/shell NaYbF4: Tm@NaYF4: Nd nanoparticles.23 After that, Prasad et al. found this concept could be used to sensitize the near infrared emission of lanthanide nanoparticles.24 In summary, dye
sensitization is an effective way to improve the luminescence intensity of LnNPs.

However, there are still some deficiencies in dye-sensitized near-infrared systems currently: (1) the sensitizer dye commonly used is a commercial dye, ICG, whose quantum yield is limited; and (2) the luminescence efficiencies of LnNPs so far reported are low, owing to multiple energy transfer steps and inevitable energy loss during the transfer process. In order to solve these problems and further improve dye-sensitized NIR lanthanide nanomaterials, efforts should be made to enhance the properties of the sensitizer dye and to optimize the energy transfer process. In the present work, we report a dye-sensitized nanomaterial consisting of a NaYbF4@NaYF4:Nd core/shell nanoparticle (denoted as CS) with organic NIR dye molecules (the carboxyl-functionalized cyanine dye derivative, Cy7) attached to the surface of CS (Scheme 1(a)). Cy7, whose quantum yield (QY) is higher than ICG, was used as the sensitizer to study the sensitization effects of organic dyes on NaYbF4@NaYF4:Nd nanoparticles. The absorption spectrum peaked at 780 nm and the emission spectrum peaked at ∼805 nm, as shown in Fig. 1(b). The spectral overlap between the emission peak of Cy7 and the absorption peak of Nd3+ will ensure that the energy absorbed by Cy7 can be effectively transferred to Nd3+. First of all, we investigated the sensitization effect in organic solvent. In order to allow the dye more convenient access to the nanoparticles, the original oleic acid ligand was removed using NOBF4, via an adapted procedure from the literature. The processed nanoparticles can be dispersed in polar solvents, such as water and ethanol. The success of ligand removal was confirmed via the measured Fourier transform infrared (FTIR) spectra. (Fig. S2†) Then, upon introducing different concentrations of Cy7 into ligand-free core/shell nanoparticles dispersed in EtOH (1 mg mL⁻¹, 1 mL), the luminescence emission intensity increased at the beginning. As the concentration increased to 4 μM, the best sensitization effect was achieved, and the optimized Cy7 concentration resulted in an approximately 15-fold enhancement in emission at 980 nm for NaYbF4@NaYF4:60%Nd (Fig. S3(a)†). The energy transfer pathways in the dye-sensitized core/shell nanoparticles are depicted in Fig. S1.† Upon continuing to increase the concentration, the emission intensity gradually declined. The observed decline can be explained through two factors: (1) increased mutual interactions between antenna molecules on the core/shell nanoparticle surfaces (self-quenching) (Fig. S4†); and (2) an increased concentration of unbound (excess) antenna molecules that absorb excitation energy but do not transfer it to the nanoparticles.

Indocyanine green (ICG) is a commonly-used dye applied to dye sensitization systems. The molecular structures of ICG and Cy7 are depicted in Fig. 1(a). Absorption spectra and emission spectra show that both have similar absorbance and emission peaks (Fig. 1(b)). However, compared with ICG, Cy7 has a higher relative quantum yield: 19.7% in EtOH. We used both ICG and Cy7 to sensitize CS nanomaterial in EtOH. In the comparison experiments, 1 mg of NaYbF4@NaYF4:60%Nd nanoparticles was dispersed in 1 mL of EtOH, and then different concentrations of Cy7 (or ICG) were introduced to the nanoparticle solution. The emission spectrum of the mixture was acquired under excitation from an 808 nm laser (2 W cm⁻²). Each time the concentration of Cy7 (or ICG)
increased, a corresponding spectrum was recorded. A series of emission spectra was acquired as the concentration of dye increased. All the test conditions were kept unchanged except for the kind of sensitizer dye. The emission spectra are shown in Fig. S3.† To compare the sensitization effects of different sensitizers, the concentration of dye is considered as variable and the intensity of the emission peak is extracted as the dependent variable. As shown in Fig. 1(c), the emission intensity of the Cy7 & CS complex at 980 nm was approximately 2.3 times that of ICG & CS. As a result, a better sensitization effect was achieved when using Cy7 as a sensitizer (Fig. 1(c)). The relative quantum yield of ICG & CS was determined to be 4.9%, but the QY of Cy7 & CS in EtOH was 6.9%, which also proved the conclusion (Fig. S12(b)†). We compared the absorption spectra of ICG & CS and Cy7 & CS complexes in EtOH and found the absorbance ability of Cy7 & CS was almost 2.8 times that of ICG & CS (Fig. 1(d)). The main reason leading to the results was the larger absorbance and high QY of Cy7.

2.2 Optimization of the acceptor

First of all, we considered selecting the structure of the nanoparticle acceptor. When Nd³⁺ ions and Yb³⁺ ions were doped together in the core without an inert shell, the emitter ions were exposed to the quenching centre, resulting in serious fluorescence quenching. However, coating with an inert shell will make energy transfer efficiency from Cy7 to Nd³⁺ relatively weak, owing to shielding from the shell. As shown in Fig. S5,† the emission intensity of Cy7 is far stronger than Yb³⁺ ions, proving that Nd³⁺ ions fail to accept excitation energy from Cy7. Therefore, we doped Nd³⁺ ions into the shell so that energy could be transferred from the sensitizer (Cy7) to emitters (Yb³⁺) via intermediate ions (Nd³⁺) efficiently. In addition, to acquire higher emission intensity from Yb³⁺ ions, we enhanced the concentration of Yb³⁺ ions to 100%. Therefore, we designed a nanomaterial with the structure NaYbF₄@NaYF₄:Nd.

Monodispersed, cubic phase core/shell NaYbF₄@NaYF₄:Nd nanoparticles were synthesized using a thermolysis procedure adapted from the literature.²⁰ Transmission electron microscopy (TEM) results (Fig. 2(b–e)) show that the NaYbF₄ core and NaYbF₄@NaYF₄:Nd core/shell nanocrystals are spherical and uniform, with mean sizes of 8.31 nm and 18.52 nm, respectively. This suggests that the shell layer has a thickness of ~5 nm in the core/shell nanoparticles. The core/shell structure was verified through high-angle annular dark-field scanning TEM (HAADF-STEM) (Fig. 2(f)). X-ray diffraction (XRD) results (Fig. S6) indicate that the core and core/shell nanocrystals are in a cubic crystallographic phase with good crystallinity.

Yb³⁺ ions usually play the role of sensitizer ions in upconversion nanoparticles, owing to their larger absorption cross section compared to other lanthanide ions.²⁵,²⁶ In our study, Yb³⁺ ions are used as emitters, mainly because of two of their unique optical properties. Firstly, the emission of Yb³⁺ ions is located at 980 nm, exactly falling into the biological optical transparent window. Secondly, there is no intermediate state between the excitation state (⁵F₅/2) and ground state (⁴F₇/2) of Yb³⁺, so luminescence quenching caused by cross relaxation can be avoided; therefore, the concentration of emitter Yb³⁺ can be set at a high level to achieve stronger emission luminescence.²⁰

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Fig. 1 (a) The molecular structures of ICG (top) and Cy7 (bottom). (b) Absorption spectra and emission spectra of ICG (top) and Cy7 (bottom) in EtOH solution. (c) The emission intensity at 980 nm from dye-sensitized nanoparticles under excitation from an 808 nm laser (2 W cm⁻²) versus Cy7 and ICG concentration, respectively. (d) Absorption spectra of Cy7 & CS and ICG & CS with the best sensitization effects in EtOH solution.
The NaYF₄ shell doped with Nd³⁺ is coated on the core, for two important reasons. Firstly, the excitation and emission energy levels of the Nd³⁺ ion are exactly located between the donating energy level of the dye and the accepting energy level of the Yb³⁺ ions, resulting in a small energy gap in the energy transfer procedure and higher energy transfer efficiency. Secondly, to a certain extent, the core/shell structure can protect the emitter through suppressing surface quenching caused by surface defects and solvents.27

In the core/shell nanoparticles, Nd³⁺ ions are donors and Yb³⁺ ions are acceptors. Since Yb³⁺ ions can efficiently receive the energy transferred from Nd³⁺ and a pure NaYbF₄ core is used as the acceptor in our design, the doping concentration of Nd³⁺ ions can be significantly enhanced, resulting in a significant enhancement in the absorption cross section of the nanoparticles.10 We adjusted the concentration of Nd³⁺ from 20% to 60% and compared the absorbance, respectively (Fig. 2(g)). The Nd doping concentration of the nanoparticles was determined via inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Fig. S7†). When the concentration increases to 80%, the nanoparticles fail to grow uniformly, owing to lattice mismatch between Nd³⁺ and Yb³⁺. A TEM image is shown in Fig. S8(a).† As the Nd³⁺ concentration increases, the absorbance of the nanoparticles increases, resulting in luminescence enhancement (Fig. S8(b)†). Then we investigated the sensitization effect in nanoparticles doped with different concentrations of Nd³⁺ ions. In comparison experiments, 1 mg of NaYbF₄@NaYF₄:x%Nd (x = 20, 40 or 60) nanoparticles was dispersed in 1 mL of EtOH. Then different concentrations of Cy7 were introduced into the nanoparticle solution. The emission spectrum of the mixture was acquired under excitation from an 808 nm laser (2 W cm⁻²). Each time the concentration of Cy7 increased, a corresponding spectrum was measured. A series of emission spectra was acquired as the concentration of Cy7 increased. All the test conditions were kept unchanged, except for the Nd³⁺ doping concentration. The emission spectra are shown in Fig. S9.† To compare the sensitization effects using nanoparticles with different Nd³⁺ doping concentrations, the concentration of dye is considered as variable and the intensity of the emission peak is extracted as a dependent variable. From the results shown in Fig. 2(h), we find that the fluorescence intensity of dye-sensitized NaYbF₄@NaYF₄:x%Nd (x = 20, 40, 60) versus Cy7 concentration under excitation from an 808 nm laser (2 W cm⁻²).

2.3 Assembly of a water soluble dye-sensitized nanocomposite

In order to attain biocompatible dye-sensitized nanocomposites for bioimaging applications, we assembled both organic dyes and core/shell nanoparticles via wrapping them in an amphiphilic phosphatidylcholine (PC) complex. The resultant micelle possesses a hydrophobic core to encapsulate the hydrophobic oleic acid coated core/shell nanoparticles through van der Waals forces and the organic dyes are inserted into the gaps between the oleic acid ligands. It also has a hydrophilic shell, offering it aqueous stability (Fig. S10(a)†). The hydrated particle size of CS & Cy7@PC is 41.5 nm, larger than the particle size (20.4 nm) of OA-CS, veri-
fying the successful assembly of PC and CS. Both of the results show that the as-synthesized micelles are well-dispersed in aqueous solution.

With an increase in Cy7 concentration, we observed that the upconversion emission intensity first increased and then decreased (Fig. S10(b)†), which was similar to the case in ethanol solution. The optimized luminescence of Cy7 sensitized CS & Cy7@PC was shown to be about 10 times greater than that of CS@PC under irradiation at 808 nm. The emission enhancement multiple is lower than that in EtOH solution, because the relative quantum yield of Cy7 in the PC cavity is reduced to 7.4% (Fig. S12(b)†). CS & Cy7@PC shows the best sensitization effect when the concentration of Cy7 is 10 μM, which is higher than that in EtOH solution. This is attributed to the dilution effect of oleic acid and PC, which alleviated the aggregation of dye molecules so that Cy7 became aggregated at a higher concentration (Fig. S11†).

2.4 Deep tissue imaging

To study the penetration depth of dye sensitized NIR nanoparticles in tissue, we added aqueous solution of CS & Cy7@PC (10 mg mL⁻¹) material to a 96-well plate, as well as a control group of CS@PC material. Then, pork tissue with different thicknesses was put in front of a NIR InGaAs camera when imaging, using excitation light from an 808 nm laser emitter (8.5 mW cm⁻²) filtered with a 950 nm long-pass optical filter. An image of CS & Cy7@PC can be distinguished by the camera at a tissue depth of 13 mm, and an emission signal can be detected at a depth of up to 16 mm (Fig. 3(a)). On the contrary, an image of CS@PC can hardly be detected at a tissue depth of 10 mm. In order to compare the difference between CS & Cy7@PC and CS@PC more intuitively, from the graph we found that NIR light emitted by CS@PC attenuated swiftly as the tissue depth exceeded 5 mm and became almost the same as background signal when the tissue depth reached 10 mm. By contrast, the emission of CS & Cy7@PC was attenuated more moderately as depth increased. These results show that dye-sensitized near infrared emission nanoparticles could be applied to deep tissue bioimaging.

2.5 Low-power excitation bioimaging

When we want to track the long-term biodistribution of nanoparticles in vivo or guide surgery with a fluorescence probe, continuous laser radiation is needed. Since high-power excitation is more likely to cause tissue damage, it is necessary to develop a fluorescence probe that can be excited under low power light. To demonstrate the capability of water-soluble dye-sensitized nanocomposites for use in low-power excitation bioimaging, we performed a lymph node imaging experiment in a nude mouse model. Water-soluble PC wrapped material (10 mg mL⁻¹, 50 μL) was used as the imaging agent and subcutaneously injected into the left hind limb. Thirty minutes later, the injected mouse was imaged with a NIR InGaAs imaging system, under excitation light from an 808 nm laser emitter with a power density of 5.0 mW cm⁻², filtered with a 950 nm long-pass optical filter. It was found that the popliteal lymph node (PO) and sciatic lymph node (SC) were lit up when injecting the mouse with dye-sensitized CS & Cy7@PC, with the signal-to-background ratio being 18.5. For comparison, we injected CS@PC at the same volume and concentration into the right hind limb of the same mouse. On the contrary, under the same power excitation light, the signal from the lymph nodes could hardly be detected in the mouse injected with CS@PC. Only when we increased the excitation light power density to 35.0 mW cm⁻² could the signal can be reluctantly distinguished from the background (Fig. 3(b)). The signal intensities of regions 1, 2 and 3 under different laser powers were extracted from the images, as shown in Fig. 3(c). Apparently, when using dye-sensitized nanoparticles as image probes, the signal can be distinguished from the background under ultra-low power excitation light.

2.6 Blood vessel imaging

Blood vessels are pipelines that deliver blood to an organism. Since many diseases can cause vascular lesions, angiography is necessary for clinical examination, to reflect the location and extent of vascular lesions accurately. To test the potential for practical application, we assessed the feasibility of CS & Cy7@PC in blood vessel imaging with a Kunming mouse model, as shown in Fig. 4(a–c). The blood vessels on the skin of the mouse’s abdomen can be clearly seen, demonstrating that our method can achieve high imaging resolution. In order to further verify the imaging resolution, we extracted the signal value along the pink dotted lines in Fig. 4(a) and (c) and attained intensity versus position curves, as shown in Fig. 4(d) and (e), sequentially, and fit them to Gaussian functions (marked with red dashed curves). Due to scattering and the absorbance of tissue, the imaging signal peak of the blood vessel will widen; the FWHM (full width at half maximum) was used to assess the width of the signal peak, which is the

![Fig. 3](image-url)
As we know, one way tumor metastasis occurs is through the lymph nodes, which is also a main factor leading to the recurrence of residual cancer. However, lymph nodes are small and complex and difficult to distinguish from muscle tissue, making lymph node dissection fairly hard. Therefore, fluorescence guidance will be useful and helpful. To prove the feasibility of using water-soluble dye-sensitized nanocomposites in fluorescence-guided surgery, we performed an experiment in a nude mouse model. Solid tumor models were established through subcutaneously injecting 50 mg of S180 tumor cells into nude mice. The tumor-bearing mice were divided into two groups: the first group was the fluorescence-guided surgery group and the second group was the control group, without guidance. Both groups of mice were injected with the dye-sensitized nanomaterial through an intratumoral injection method. Owing to lymphatic circulation, the nanomaterial will be stranded in the lymph nodes and will then light the lymph nodes up under excitation light. The first group of mice was imaged with the NIR InGaAs imaging system under the same imaging conditions as mentioned above (Fig. 5(b)). Before surgery, the lymph node could hardly be distinguished under the imaging camera, due to the fluorescence of the tumor. After the removal of the tumor, the margin of the lymph node became much clearer and could even be seen without opening the skin. With the guidance of fluorescence, the peritumoral lymph node was successfully dissected from the mouse, with no residual tissue. The pathological section also proved the accurate removal of the lit lymph node (Fig. 5(c)). On the contrary, without the guidance of fluorescence, it is difficult to distinguish lymph nodes from muscle tissue (Fig. 5(d)).

3 Materials and instruments

3.1 Materials

All chemicals used were of analytical grade and were used without further purification. Deionized water was used throughout. Oleic acid (OA: >90%), 1-octadecene (ODE: >90%) and oleylamine (OM: >90%) were purchased from Sigma-Aldrich. CF3COONa (>99%) was purchased from Acros. Rare-earth oxides, RE2O3 (99.999%) (RE3+ = Y3+, Yb3+, Nd3+), were purchased from Shanghai Yuelong New Materials Co. Ltd. Trifluoroacetic acid was bought from Sinopharm Chemical Reagent Co., China. Ethanol and cyclohexane were purchased from Adamas-beta Reagent. Nitrosomonium tetrafluoroborate (NOBF4) was bought from Alfa Aesar Ltd. Ln(CF3COO)3 (Ln = Yb, Y, Nd) was prepared through dissolving the corresponding rare earth oxide in trifluoroacetic acid solution with continuous stirring at elevated temperature to form a transparent solution and then evaporating the water completely.

3.2 Characterization

X-ray powder diffraction (XRD) pattern measurements were made using a Bruker D4 diffractometer at a scanning rate of 1° min⁻¹ in the 2θ range from 10° to 90° (Cu Kα radiation, λ = 0.15406 nm). The sizes and morphologies of nanoparticles were characterized at 200 kV using a FEI Tecnai G2 20 Twin TEM. FTIR spectra were obtained using a ThermoFisher Nicolet 6700 spectrometer from samples in KBr pellets. UV-vis absorption spectra were recorded using a Shimadzu 3000 spectrophotometer. NIR fluorescence spectra were recorded using an Edinburgh Instruments FLS920 fluorescence spectrometer, equipped with an external 0–10 W adjustable laser (808 nm, Beijing Hi-Tech Optoelectronic Co., China) as the excitation source at room temperature. The collection range was from 950 nm to 1100 nm. Dynamic light scattering (DLS) measurements were taken using a Malvern nanoparticle size-zeta potential analyzer. 1H NMR and 13C NMR spectra were recorded on a Bruker spectrometer at 400 MHz. Matrix assisted laser desorption ionization-time of flight mass spectra (MALDI-TOF-MS) were obtained using an AB SCIEX 5800 system. All chemical shifts are reported in standard δ notation of parts per million. Elemental analysis was conducted using a ThermoFisher iCAP 7400 ICP-AES. All NIR bioimaging pictures were taken using a Princeton InGaAs NIR CCD.
3.3 Synthesis of oleated-capped NaYbF₄@NaYF₄:Nd (denoted as OA-CS)

The synthesis of the NaYbF₄ core is via the co-thermolysis of trifluoroacetates.²⁸ 1 mmol of CF₃COONa and 1 mmol of Yb(CF₃COO)₃ were added to a three-necked flask containing 20 mmol of ODE, 10 mmol of OA and 10 mmol of OM at room temperature. The mixture was heated at 110 °C under vacuum with magnetic stirring until a transparent solution was formed. Then the solution was heated to 300 °C under nitrogen protection and maintained for 30 min. After being cooled down to room temperature, the products were collected via centrifugation at 15 000 rpm for 10 min. The as-prepared nanoparticles were dispersed in cyclohexane (10 mL).

OA-NaYbF₄@NaYF₄:Nd nanoparticles were synthesized through epitaxial growth. Then 1 mmol of as-prepared nanoparticles, 1 mmol of RE(CF₃COO)₃ and 1 of mmol Na(CF₃COO) were added to a three-necked flask containing 20 mmol of ODE and 20 mmol of OA. The solution was stirred for 30 min at 90 °C to evaporate cyclohexane. The mixture was then heated to 300 °C under nitrogen protection and kept for 30 min. After cooling down to room temperature, the products were collected via centrifugation at 15 000 rpm for 10 min. Finally the as-prepared core/shell nanoparticles (OA-CS) were dispersed in cyclohexane (10 mL).

3.4 Synthesis of Cy7

The synthetic procedure is depicted in Fig. S13.† Synthesis of compound 1: 2,3,3-trimethylindolenine (6.3 g, 62 mmol) and 3-bromopropionic acid (8.2 mL, 94 mmol) were dissolved in toluene (50 mL), and the solution was heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature and the resulting crystals were filtered and washed with acetone. The filtered product was recrystallized from a solution of MeOH and Et₂O. The crystals were collected and dried under vacuum. Synthesis of compound 2: A solution of POCl₃ (37 mL, 397 mmol) in DCM (35 mL) was slowly added to an ice-cooled solution of DMF (40 mL, 516 mmol) in DCM (40 mL). After the addition was finished, cyclohexanone (10 g, 100 mmol) was added via a syringe. The resulting reaction mixture was refluxed for 2 h. The mixture was then cooled in
ice. Water (200 mL), pre-cooled to 0 °C, was added slowly while the mixture was stirred. Then the mixture was stirred for 30 min. The DCM layer was collected and the water layer was extracted with additional DCM. The DCM solutions were combined, passed through an MgSO4 column, concentrated on a rotavap and treated with pentane (200 mL) to give compound 2 as a yellow crystalline solid, to be reserved at cool temperatures. Synthesis of Cy7: Compound 1 (7.9 g, 20 mmol), freshly prepared compound 2 (1.7 g, 10 mmol), n-butanol (200 mL) and benzene (20 mL) were added into a flask with a Dean-Stark trap and condenser attached. The mixture was heated to 120 °C for 24 h, resulting in a green solution. Solvents were removed on a rotavap.

3.5 Preparation of dye-sensitized nanoparticles (CS & Cy7)

Firstly, ligand-free NaYbF4@NaYF4:60%Nd nanoparticles were prepared through NOBF4 treatment, according to the previous literature.29 Typically, the as-synthesized oleate-capped core/shell nanoparticles (20 mg) were dispersed in cyclohexane and 4 mL of a dichloromethane solution of NOBF4 was added. The mixture was ultrasonicated for 3 min to remove the surface ligands. After that, the nanoparticles were collected via centrifugation at 15 000 rpm for 10 min. The prepared products were dispersed in EtOH. Cy7 dye-sensitized nanoparticles were prepared through mixing different amounts of Cy7 into the ligand-free nanoparticles in EtOH.

3.6 Synthesis of phosphatidylcholine encapsulated dye-sensitized nanocomposite (OA-CS & Cy7@PC)

OA-CS (10 mg) was dispersed in 5 mL of CH2Cl2 via ultrasonication, then Cy7 was added, and the mixture was stirred at room temperature to obtain a homogeneous phase. Furthermore, amphiphilic phosphatidylcholine (10 mg) was added, and then the mixture was stirred overnight at room temperature. The mixture was centrifugated, and the collected solid was repeatedly washed with water. The precipitate could be redispersed in deionized water.21

3.7 NIR imaging system and data processing software

NIR luminescence imaging was performed using a NIR in vivo imaging system designed by our group. We used an 808 nm laser as the excitation source and a Princeton InGaAs CCD as a signal collector. The excitation intensity of the 808 nm irradiation was kept below 50 mW cm\(^{-2}\) and the exposure time was 100 ms. The signals were collected at wavelengths larger than 950 nm. Images of luminescent signals were analysed using Carestream MI SE and AndorSolis.

3.8 Tissue depth penetration

200 μL of CS & Cy7@PC (10 mg mL\(^{-1}\)) and CS@PC (10 mg mL\(^{-1}\)) aqueous solution were added into two different holes of a 96-well plate. NIR imaging of both holes was conducted under pork tissue of increasing thickness.

3.9 Low-power excitation imaging

All the animal procedures were carried out in accordance with the guidelines of the National Institute for Food and Drug Control, China, and were approved by the Institutional Animal Care and Use Committee (IACUC), School of Pharmacy, Fudan University. 4-Week old nude mice were used for lymph node imaging experiments. 50 μL of CS & Cy7@PC (10 mg mL\(^{-1}\)) aqueous solution was injected into a nude mouse from the left hind limb and 50 mL of CS@PC (10 mg mL\(^{-1}\)) aqueous solution was injected from the right hind limb. Half an hour after injection, the mouse was injected with chloral hydrate solution for anesthetization. The excitation intensity of 808 nm irradiation was varied from 5.0 mW cm\(^{-2}\) to 49.0 mW cm\(^{-2}\).

3.10 Blood vessel imaging

A 4-week old Kunming mouse was used for blood vessel imaging experiments. 200 μL of CS & Cy7@PC (5 mg mL\(^{-1}\)) aqueous solution was injected into the nude mouse from the tail vein. The in vivo images were shot 5 minutes after injection.

3.11 Fluorescence-guided peritumoral lymph node dissection

Animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee. About 50 μL of S180 cells (3 × 10\(^6\) cells per mL) were grafted into a nude mouse. Tumors with diameters of around 12 mm formed after 2 weeks. 300 μL of CS & Cy7@PC (5 mg mL\(^{-1}\)) aqueous solution was injected into the tumor-bearing mouse through intratumoral injection. The tumor resection and lymph node dissection surgery was conducted 3 hours after injection.

4 Conclusions

In conclusion, we have found a more efficient dye-sensitized NIR lanthanide nanomaterial through using NaYbF4@NaYF4:60%Nd as an acceptor and replacing the traditional ICG with a higher-QY sensitizer, Cy7. Through using a pure NaYbF4 emitter core, we increased the Nd\(^{3+}\) doping concentration up to 60%, which was higher than the concentration of 30% in most reported dye sensitization systems, and significantly increased the absorbance of the nanoparticles. On the other hand, by using Cy7 as a sensitizer, we acquired dye-sensitized lanthanide nanomaterials with a QY of up to 6.9% in EtOH, which was better than using traditional ICG. The optimized Cy7 & CS complex was then wrapped with phosphatidylcholine to form a water-soluble and biocompatible material. This NIR emission material was applied to guide peritumoral lymph node dissection and was demonstrated to be a promising imaging agent for fluorescence-guided surgery.

Conflicts of interest

There are no conflicts to declare.
Notes and references


Acknowledgements

The authors thank the National Natural Science Foundation of China (21722101, 21671042, and 21527801), the National Key R&D Program of China (2017YFA0205100), the National Basic Research Program of China (2015CB931800), and Shanghai Sci. Tech. Comm. (15QA1400700) for financial support. The authors also thank Yawei Liu, Xianlong Su and Ti Jia from Fudan University for support in the synthesis and characterization of the organic dye.