Energy Transfer Highway in Nd$^{3+}$-Sensitized Nanoparticles for Efficient near-Infrared Bioimaging

Cong Cao, Meng Xue, Xingjun Zhu, Pengyuan Yang, Wei Feng,* and Fuyou Li*©

Department of Chemistry & Institute of Biomedicine Sciences & State Key Laboratory of Molecular Engineering of Polymers & Collaborative Innovation Center of Chemistry for Energy Materials, Fudan University, 220 Handan Road, Shanghai, P.R. China

Supporting Information

**KEYWORDS:** Nd$^{3+}$-sensitized, NIR probe, energy transfer, optimization luminescence, high spatial resolution, bioimaging

**INTRODUCTION**

Fluorescence-based biological imaging attracts more and more attention for diverse applications in clinical diagnosis and therapy because of its high spatial resolution and fast sensitivity.¹⁻⁴ The concept of tissue transparent window in near-infrared region (NIR, 700–1700 nm) has been proposed recently, which exhibits so many merits: deep tissue penetration, low scattering and absorption of light, weak autofluorescence from biological organisms, etc.⁵⁻⁹ Different from other luminescence materials (e.g., carbon nanomaterials, quantum dots, fluorescent dyes),¹⁰⁻¹¹ lanthanide-doped luminescence nanocrystals are promising probes because of their outstanding photochemical properties, including narrow emission bands, relatively low toxicity, and superior photo-stability.¹²⁻¹⁵ Previous research has focused on upconversion nanoparticles (anti-Stokes shift emission) for biomedical imaging,¹⁶⁻¹⁹ whereas Nd$^{3+}$ or Er$^{3+}$ ions doped nanocrystals with large Stokes shift emission are also ideal NIR probes and need more investigation.¹⁵,²⁰⁻²⁸ However, the doping concentration of rare earth activator ions is largely restricted by drastic cross-relaxation. In traditional Nd$^{3+}$ doped luminescence probe, the optimal doping amount is only around 5%.²²⁻²⁸ The concentration quenching impedes the system effectively harvesting more excitation light and thus obtains limited emission light, which would be unfavorable for luminescence probes.

High-efficiency energy transfer (up to 70%) in Nd$^{3+}$-sensitized Yb$^{3+}$ system has been reported in many kinds of host materials.²²,²⁹⁻³⁷ The sensitizing process makes it possible for elevating Nd$^{3+}$ doping amount.²² However, Nd$^{3+}$ and Yb$^{3+}$ ions were always codoped with other activators (Er$^{3+}$, Tm$^{3+}$, Ho$^{3+}$, Tb$^{3+}$) to optimize upconversion luminescence rather than NIR emission. Moreover, the doping concentration of Nd$^{3+}$ was still restricted to utilizing the excitation energy. Therefore, we just introduce Yb$^{3+}$ ions into Nd$^{3+}$ doped system without other activators, to construct an energy transfer highway for promoting the energy utilization efficiency. On this transfer highway, Yb$^{3+}$ ions acted as the only activators for NIR emission, which can quickly receive energy from Nd$^{3+}$ ions. Thus, it can reduce the amount of excited-state-Nd$^{3+}$ in the luminescence process, and store much excitation energy for the following luminescence process.²²,³⁰⁻³²,³⁴,³⁶ The energy transfer highway will give rise to increasing the doping concentration of Nd$^{3+}$ and simultaneously enhancing the NIR emission. Based on this concept, we designed an Nd$^{3+}$-sensitized-Yb$^{3+}$ system to maximize the energy utilization efficiency. In addition, surface-related NIR quenching in the aforementioned nanosystem may be significantly suppressed by the core/shell structure just like upconversion luminescent nanoparticles.¹³,¹⁴,¹⁷,³⁸,³⁹

After luminescence optimization, cubic phase of NaYF$_4$:7% Yb,60%Nd@CaF$_2$ nanocrystals, with small size (~12 nm) and high quantum yield (~20%) were finally synthesized. The high efficiency of energy transfer in this Nd$^{3+}$-sensitized-Yb$^{3+}$
system, exciting at 808 nm, providing NIR emission at 980 nm, which would be a promising candidate for biological imaging. Ethylenediamine tetramethylenephosphonic acid (EDTMP) (Figure S1) was further modified on the nanoparticles to improve the water-solubility and extend blood circulation time. Two V-shaped capillaries can be distinguished through 10 mm pork tissue. Finally, an in vivo vascular imaging in the mouse hindlimb with high spatial resolution down to 0.19 mm at a depth of 2–3 mm was detected clearly.

## RESULTS AND DISCUSSION

To optimize energy transfer between Nd$^{3+}$ and Yb$^{3+}$ ions, we chose cubic phase NaREF$_4$ and CaF$_2$ outer layer to construct classical and distinguishing core/shell structure (Figure 1a). We synthesized NaYF$_4$:x%Yb$_{y%}$Nd$_{z%}$NPs by a thermolysis method. The transmission electron microscopy (TEM) images (Figure 2a, Figures S2 and S3) showed that all prepared NaYF$_4$:x%Yb$_{y%}$Nd NPs have the spherical shape ~6 nm in diameter. The dopant of Nd$^{3+}$ ions did not change the shape and phase of the nanoparticles (Figure S2). It was verified by the X-ray powder diffraction (XRD) measurement that the core was cubic phase of NaNdF$_4$ (Figure S4). Meanwhile, the high-resolution transmission electron microscopy (HRTEM) image (Figure 2b) was in agreement with XRD pattern. A small shift of the diffraction peaks compared with standard pattern of cubic phase NaNdF$_4$ (JCPDS: 27−0757) was due to the larger ionic radius of Nd$^{3+}$ than Y$^{3+}$ ions.

TEM images of the oleic acid capped NaYF$_4$:7%Yb$_{60%}$Nd@CaF$_2$ core/shell nanoparticles (OA-CSNPs) showed that the diameter of OA-CSNPs was 12 nm and the thickness of the CaF$_2$ layer was 3 mm (Figure 2d). In addition, the little diffraction peak centered at 32° of NaYF$_4$:Yb$_{60%}$Nd core nanoparticle can be attributed to the cubic phase of NaNdF$_4$, indicating the difference between standard cubic phase of NaNdF$_4$ and standard cubic phase of CaF$_2$ (JCPDS: 35−0816) (Figure S4 and Figure 2c). After coating the thick CaF$_2$ shell, the little peak centered at 32° of NaYF$_4$:Yb$_{60%}$Nd@CaF$_2$ (content ratio = 1:4) core/shell NPs was not obvious. Meanwhile, the HRTEM image (Figure 2e) was in agreement with XRD pattern. The core/shell structure was quiet discernible under high-angle annular dark-field scanning TEM (HAADF-STEM) (Figure 2f). The energy dispersive X-ray analysis (EDXA) also demonstrated the successful synthesis of the OA-CSNPs, in which Ca was coexist with other elements (Figure S5).

As a contrast, the Nd$^{3+}$-based NPs was first synthesized, in which Nd$^{3+}$ acted simultaneously as the sensitizer and emitter. Conventional small and unusual large amounts of Nd$^{3+}$ ions were separately doped into cubic phase NaYF$_4$ NPs. There are three NIR absorption peaks, which located at 740, 800, and 860 nm, corresponding to transition from $^{4}I_{9/2}$ to $^{2}F_{7/2}, ^{2}F_{5/2}$, and $^{2}F_{3/2}$ states (Figure 1b). NIR emissions peak of Nd$^{3+}$ ions were separately at 860 nm ($^{2}F_{5/2}$ → $^{4}I_{9/2}$), 890 nm ($^{2}F_{7/2}$ → $^{4}I_{11/2}$), 1060 nm ($^{2}F_{5/2}$ → $^{4}I_{13/2}$) and 1330 nm ($^{2}F_{3/2}$ → $^{4}I_{15/2}$) (Figure 1c). Notably, the integrated absorption at 800 nm of NaYF$_4$:60%Nd was about 17 times higher than the optimized NaYF$_4$:5%Nd (Figure 1b), while the luminescence was lower than the latter (Figure S6a). It can be seen that the optimal doping amount of Nd$^{3+}$-based system was surely about 5%, coincide with traditional reports. Higher doping concentration truly caused deleterious concentration quenching, weak luminescence, and low energy utilization efficiency. Considering concentration quenching in Nd$^{3+}$-based NPs, large absorption cross-section of Nd$^{3+}$ ions and high Nd$^{3+}$ → Yb$^{3+}$ energy transfer efficiency make Nd$^{3+}$-sensitized-Yb$^{3+}$ system more feasible in enhancing the utilization of excitation energy and overcoming concentration quenching. We introduced Yb$^{3+}$ ions as activators to construct Nd$^{3+}$-sensitized photoluminescence system. The designed structure of NPs and proposed energy transfer pathway for this sensitized system were shown in Figures 1a and 3a. A decrease in emission peak from Nd$^{3+}$ and simultaneous appearance of peak at 980 nm from Yb$^{3+}$ were observed in NaYF$_4$:Yb$_{60%}$Nd nanoparticles (Figure 3b, e and Figure S6). This observation also illustrated that Nd$^{3+}$ effectively transferred energy to the Yb$^{3+}$ ions.
The doping concentration adjustment was investigated to achieve the maximum NIR emission intensity. Activators were usually at low concentration; therefore, we fixed the Yb$^{3+}$ ion dopant concentration at 7% (Figure 3b). In comparison with activators, Nd$^{3+}$ ions served as light absorber and sensitizers, and the doping concentration was regulated over a large range (from 30% to 95%). We observed that the luminescence intensity was also increased along with the Nd$^{3+}$ doping amount increasing. It means that much more energy is absorbed from the excitation light by Nd$^{3+}$ ion dopant elevating. Nevertheless, more sensitizer doping would lead to deleterious cross-relaxation, and thus the emission was weakened with Nd$^{3+}$ doping concentration increasing right alongside (Figure 3e and Figure S6c). On this occasion, Nd$^{3+}$ ions doping concentration was finally determined at 60%, which issued the brightest NIR emission.

The luminescence mechanism was proposed as a single photon process in the energy transfer highway. To determine the mechanism, we detected NIR luminescence of nano-particles under gradually increased excitation power density. The 808 nm laser excited Nd$^{3+}$ ions to its 4F5/2 state, followed by a nonradiation transition to its 4F3/2 state, and then the energy transferred to the state 2F5/2 of Yb$^{3+}$, then quickly emitting at 2F7/2 (Figure 3a). In theory, the luminescence emission is proportional to the increased exciting pump power density. The results revealed its truly a single photon process (Figure S7).

It should be noted that both the absorption and near-infrared emission of Nd$^{3+}$-sensitized-Yb$^{3+}$ system are enhanced by contrast with only Nd$^{3+}$ ions doped system. The integrated absorption peak centered at 800 nm for Nd$^{3+}$ was about 25 times larger than the absorption peak at 980 nm for Yb$^{3+}$ ions in the OA-CNPs (Figure 1b). The integrated emission peak at 980 nm for OA-CNPs was ~8 times higher than that at 1060 nm for NaYF4:60%Nd3+ even though they have almost same absorption intensity at 800 nm (Figure 1c). In contrast, there was no obvious NIR emission from NaYF4:7%Yb NPs under 808 nm excitation. Above results implied the feasibility of using Nd$^{3+}$-sensitized NPs which rendered NIR luminescence surpassing the conventional Nd$^{3+}$-based NPs. This conclusion is supported by the excitation spectra from the state 4F7/2 of Yb$^{3+}$ ions in OA-CNPs (Figure S8). The observed excitation peaks matched with three NIR absorption peak of Nd$^{3+}$ ions at 740, 800, and 860 nm and one peak of Yb$^{3+}$ ions at 980 nm. CaF$_2$ optical innert layer was coated to protect inner sensitizer and activator from surface quenching. The growth of CaF$_2$ shell on OA-CNPs suppressed nonradiative transition at the nanoparticle surface and reduced surface defects. The integrated NIR luminescence intensity from the OA-CSNPs was 2.5 times higher than that of OA-CNPs (Figure 4a) under excitation of an 808 nm laser. Furthermore, CaF$_2$ shell (rare earth ions free) could depress RE ionic leakage to reduce the potential toxicity in biomedical applications.29,39

To further certify the effect of core/shell structure, we measured the lifetime of Nd$^{3+}$ and Yb$^{3+}$ ions, respectively. When the CaF$_2$ shell was applied to NaYF$_4$:7%Yb,60%Nd NPs, the lifetime of Yb$^{3+}$ ($^2$F$_{5/2} \rightarrow ^2$F$_{7/2}$) was recorded from 52 µs increased to 251 µs (Figure 4d). Although the lifetime of Nd$^{3+}$...
ions (4F3/2 → 4F9/2) has a slight change after coating with CaF2 (from 31 to 22 μs, Figure 4c). It suggested that the CaF2 shell could effectively prevent surface quenching mainly from Yb3+ ions and little surface quenching from Nd3+ ions. Both enhanced emission intensity and lengthened lifetime of Yb3+ in core/shell nanoparticles verified the protective effect of CaF2.

The absolute quantum yield (QY) was defined as the ratio of the quantity of emitted photons to the quantity of absorbed photons, determined to be 20.7% for OA-CSNPs, under a high energy power xenon lamp (150 W) at the excitation of 808 nm. As we all known, absorbance and quantum efficiency are the determinants of fluorescence emission. This new luminescent probe has significantly enhanced absorption band and high quantum yield, and work in the near-infrared window, indicating the high energy utilization efficiency and potential application as a sensitive biomedical imaging probe.

To validate the in vitro and in vivo biological imaging performance, the OA-CSNPs need to be transferred to the hydrophilic phase. EDTMP was coated to replace the surface oleic acid ligand of the NPs, forming EDTMP-modified NaYF4:7%Yb,60%Nd nanoparticles (called EDTMP-CNP(7)) and EDTMP-modified NaYF4:7%Yb,60%Nd@CaF2 nanoparticles (EDTMP-CSNPs) by using a ligand exchange method.11,42 The luminescence of EDTMP-CNP(7) was only 20% of the OA-CSPs because of quenching in water. Here, CaF2 inert layer isolated inner sensitizer and activator from surface quencher in solution, so that EDTMP-CSNPs remained 70% luminescence of the OA-CSNPs (Figure 4a), which is another great demonstration of the protective effect of CaF2 shell.

Next, we investigated cytotoxicity of EDTMP-CSNPs through a methyl thiazolyl tetrazolium (MTT) assay experiment. The biocompatibility of the EDTMP-CSNPs was carried on Hela cells after incubation with different concentrations of EDTMP-CSNPs (0, 50, 100, 200, 400, 800 μg/mL) for 24 h. The MTT results (Figure S12) suggested that Hela cells remained good viability under different concentrations of NPs.
Regarding to the potential toxicity, we further investigated the histology test. No obvious lesion was detected, revealing that no noticeable toxicity to mice (Figure S13).

The frightful challenges of in vivo imaging are the limited tissue penetration depth and poor resolution. They are closely related to the complicated biological environment and affected by the photon absorption and scattering of tissue. Here, we designed an in vitro simulation experiment to evaluate tissue penetration and spatial resolution systematic (Figure 5). Pork tissue with different thickness (0, 5 mm and 10 mm) was separately covered onto two V-shaped capillaries (0.50 mm inner diameter) filled by EDTMP-CSNPs solution. The capillaries were then illuminated with an 808 nm laser, whereas the luminescence signals were collected by the camera. It is noteworthy that the detection QY of the camera is about 20% at 980 nm (Figure S14). As shown in Figure 5c, the V-shape of the capillaries was detectable unambiguously with sharp peaks even under 5 mm pork slices; meanwhile, the calculated diameter (0.50 mm inner diameter) filled by EDTMP-CSNPs solution. The capillaries were then illuminated with an 808 nm laser, whereas the luminescence signals were collected by the camera. It is noteworthy that the detection QY of the camera is about 20% at 980 nm (Figure S14). As shown in Figure 5c, the V-shape of the capillaries was detectable unambiguously with sharp peaks even under 5 mm pork slices; meanwhile, the calculated diameter (0.50 mm inner diameter) also matched well with the actual diameter, demonstrating high resolution even under 5 mm tissue. The V-shaped capillaries were still visible through 10 mm pork tissue, but the broad peaks showed weak resolution, reflecting absorbance, and scattering of photons by the tissue (Figure 5e).

An in vivo imaging experiment was conducted by intravenously injecting EDTMP-CSNPs. The liver of the nude mouse can be observed clearly after injection within 1 min (Figure 6a). The luminescence intensity profiles of blood vessels were shown in Figure 6, in which peaks could be identified and fit to Gaussian functions (marked with red dash curves). Here, the FWHM was used for estimating the width of the vessels. Femoral arteries in vivo imaging were distinguished unambiguously with a diameter of 0.19 mm (Figure 6h). The penetration depth of femoral arteries was measured to be about 2–3 mm by subsequent dissection experiment. The calculated values of other blood vessels width extracted from luminescence image were 0.26 mm, 0.42 mm and 0.54 mm, agreed well with the in situ bright-field picture (Figure 6g, i). This NIR imaging was performed on our EMCCD camera with a pixel size of 0.13 mm in the view without additional high magnification objective lens. These results proved that the EDTMP-CSNP can realize visualization of a small blood vessel, which will be promising for vascular-related disease imaging and angiogenesis diagnosis in tumors.

**CONCLUSIONS**

In conclusion, we synthesized Nd³⁺-sensitized-Yb³⁺ core/shell nanoparticles, which have an average diameter of 12 nm, strong NIR emission at 980 nm and high quantum yield up to 20.7%. Nd³⁺ ions acted as activators, with the increased amount (60%) making it absorb more excitation energy at 808 nm, whereas Yb³⁺ ions manifest energy transfer highway effect. After coating with CaF₂ inert shell and ligand exchange, the water-soluble EDTMP-CSNPs showed bright NIR luminescence, good biocompatibility, and low biotoxicity. Two V-shaped capillaries can be distinguished clearly even through 10 mm pork tissue, showing deep penetration. Finally, an in vivo vascular imaging spatial resolution down to 0.19 mm in the mouse hindlimb was obtained, demonstrating that NaYF₄:7%Yb,60%Nd@CaF₂ core/shell nanocrystal can be used as an efficient NIR probe.
**EXPERIMENTAL SECTION**

**Materials.** Lanthanide oxides, Y$_2$O$_3$ (>99.999%), Nd$_2$O$_3$ (>99.999%), and Yb$_2$O$_3$ (>99.999%) were all brought from Shanghai Yuelong Rare Earth New Materials Co., Ltd. Trifluoroacetic acid sodium salt (CF$_3$COONa, 99%), oleylamine (OM; > 90%), oleic acid (OA; > 90%), and 1-octadecene (ODE; > 90%) were always bought from Sigma-Aldrich Co.. Ethylenediamine tetra (methylene phosphonic acid) (EDTMP) were purchased from TCI (Shanghai) Development Co., Ltd. CaCO$_3$, cyclohexane solvent, ethanol solvent and trifluoroacetic acid (TFA) were always brought from Sinopharm Chemical Reagent Co., China. Ln(CF$_3$COO)$_3$ solid powders were all obtained through corresponding oxide reacting with excessive TFA. Ca(CF$_3$COO)$_2$ was obtained by CaCO$_3$ reacting with excessive TFA. The chemical reagents used in the experiments were all of analytical grade with no further purification. In all experiments, deionized water was used.

**Characterization.** TEM images, HRTEM images, HAADF-STEM images, and EDX spectra of the as-prepared nanocrystals were determined by a Tecnai G2 F20 S-Twin (FEI, America) operating at 200 kV. XRD patterns were carried out on D8 advance diffractometer (Bruker, Germany) with a scanning rate of 0.5° per minute by using Cu Kα radiation (λ = 0.15406 nm). Absorption spectrum was measured with Lambda 750 (PerkinElmer, America). The NIR photoluminescence spectrum were measured on QM 40 (PTI, America) with an external 808 nm continuous wavelength (CW) laser. The photostability of EDTMP-CSNPs and ICG dyes are measured by continuously excited by an 808 nm CW laser (~200 mW/cm$^2$) for 30 min. The quantum yield of OA-CSNPs powder was measured on Quantaurus-QY Plus from Hamamatsu Co. with a high-energy xenon lamp (150 W) as excitation source. We characterized the dynamic light scattering (DLS) and zeta-potential of the NPs on ZS90 (Malvern, England). An InGaAs CCD camera from Hamamatsu Co. collected the NIR luminescence photographs under an 808 nm CW laser (~200 mW/cm$^2$).

**Synthesis of NaYF$_4$:x%Yb,y%Nd NPs Capped with OA.** In the case of NaYF$_4$:x%Yb,y%Nd NPs: 1 mmol CF$_3$COONa, x% mmol of Yb(CF$_3$COO)$_3$, x% mmol of Nd(CF$_3$COO)$_3$, and (100-x-y)% mmol of Y(CF$_3$COO)$_3$, were mixed into a 100 mL three-neck flask containing OM (10 mmol), OA (10 mmol), and ODE (20 mmol). The mixed solution was heated to 130 °C under vacuum for several minutes with continuous stirring until the mixture turned to transparent. Then the transparent solution was heated to 310 °C and kept for 45 min under nitrogen protecting. The nanoparticles were precipitated by adding much ethanol and centrifuging, and finally dispersing in 10 mL of cyclohexane for further application.

Figure 6. (a) In vivo NIR imaging was collected by intravenous injecting EDTMP-CSNPs (15 mg/kg per nude mouse). (b) In vivo imaging of the bright field was displayed. (c) In vivo NIR imaging was collected after mouse liver covered. (d, f) In situ NIR imaging of the mouse was collected. (e) In situ imaging of the bright field was collected. (g–i) Cross-sectional fluorescence intensity profiles corresponding to the white dash lines marked in d, c, and f, respectively. Red dashed curves represented Gaussian functional fitting lines and the corresponding FWHM was displayed.
Synthesis of NaYF₄:7%Yb,60%Nd@CaF₂ Core/shell NPs Capped with OA (OA-CSNPs). The 0.5 mmol of as-prepared optimized NaYF₄:7%Yb,60%Nd core nanoparticles and 2 mmol Ca(CF₃COO)₂ powder were added into a 100 mL three-neck flask containing with ODE (20 mmol) and OA (20 mmol). The mixed solution was heated to 110 °C to remove cyclohexane with constant stirring under vacuum until formed a transparent mixture. Then the solution was heated to 310 °C and kept for 30 min under nitrogen protecting. The OA-CSNPs were precipitated by adding much ethanol. Finally, the products were dispersed in 5 mL of cyclohexane.

Synthesis of NaYF₄:7%Yb,60%Nd@CaF₂ NPs Modified with EDTDMP. The 0.2 mmol of OA-CSNPs was added with excessive dichloromethane solution of NOBF₄ at room temperature. The precipitated nanoparticles was dispersed in water after removing the supernatant by centrifugation. Ethanol and deionized water were added to further purify the dispersion. Then ligand-free nanoparticles were dispersed in EDTMP solution (50 mg/mL) for 30 min with stirring, washed with water, and collected by centrifuging.

Cytotoxicity Assay. The in vitro cytotoxicity was performed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Human epithelial adenocarcinoma Hela cells were incubated with different concentrations of EDTMP-CSNPs (0, 50, 100, 200, 400, 800 μg/mL) in a 96 well cell culture plate for 24 h with a constant temperature at 37 °C under 5% CO₂ atmosphere. Subsequently, 10 μL MTT (5 mg/mL) solution was separately added to every well and then incubated for 4 h. After removing the medium and adding dimethyl sulfoxide solution (DMSO, 100 μL) to each well, a Tecan Infinite M200 monochromator was used to collected the optical density OD570 value (Abs.) of per well with background subtraction at 690 nm. The formula for calculating viability of cell growth is shown as follows:

\[
\text{cell viability} (\%) = \left( \frac{\text{mean of Abs. of treatment groups}}{\text{mean of Abs. of control groups}} \right) \times 100
\]

Histological Assessment. All animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee, Department of Pharmacy, Fudan University. In the test group, three four-week-old BALB/c female mice were all intravenously injected with EDTMP-CSNPs (0.3 mg). Moreover, three BALB/c female mice with no injection were set as the blank group. Tissue samples were harvested from test group and control group after 7 days. Both groups of mice were all sacrificed, and the liver, spleen, lung, heart, and kidney of the mice were collected and immersed in paraformaldehyde, then embedded into paraffin, finally sectioned, and stained by hematoxylin and eosin for further observation.

In Vitro and in Vivo NIR Luminescence Bioimaging. All mouse luminescence imaging experiments were carried out in the in vivo luminescence imaging system reported by our group. To determine penetration depth and resolution of EDTMP-CSNPs in NIR luminescence bioimaging, we tested the luminescence signals of the two capillaries of V-shaped arrangement filled with the EDTMP-CSNPs (2.0 mg/mL in water) under different thickness of pork (0 mm, 5 mm, 10 mm). We used an 808 nm CW laser as excitation source (~200 mW/cm²); meanwhile, a 980 nm long-pass filter from Semrock Co. was used to collected signals in front of an Andor DU987 EMCCD camera (512 × 512-pixel silicon charge-coupled device) for NIR imaging. Then EDTMP-CSNPs (2.0 mg/mL, 0.20 mL) were injected intravenously into a nude female mouse for imaging. The in vivo bioimaging experiment was done with the same conditions with the capillaries imaging experiment. Signals obtained from luminescent images were all analyzed with Andor Sofware, Bruker Molecular Imaging Software (Bruker MI SE) and Origin 8.0 software.

**REFERENCES**


**AUTHOR INFORMATION**

Corresponding Authors

*E-mail: fengweis@fudan.edu.cn.* (W.F.); *E-mail: fyli@fudan.edu.cn.* (F.Y.L.).

ORCID

Wei Feng: 0000-0002-8096-2212

Fuyou Li: 0000-0001-8729-1979

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors are very grateful for the NIR photoluminescent lifetime measurements from Professor Xueyuan Chen and Doctor Dato Tu, in Fujian Institute of Research on the Structure of Matter (Chinese Academy of Sciences, CAS). The authors acknowledge the financial support from National Basic Research Program of China (2015CB931801, 2013CB733703), National Natural Science Foundation of China (21527801, 21527801, 21527801, 21527801); Shanghai Sci. Tech. Comm. (15QA1400700) and the CAS/SSAFEA International Partnership Program for Creative Research Teams.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsaami.7b04305.