Intense near-infrared-II luminescence from NaCeF₄:Er/Yb nanoprobes for in vitro bioassay and in vivo bioimaging†

Xialian Lei, Renfu Li, Datao Tu, Xiaoying Shang, Yan Liu, Wenwu You, Caixia Sun, Fan Zhang and Xueyuan Chen

Near-infrared (NIR) II luminescence between 1000 and 1700 nm has attracted reviving interest for biosensing due to its unique advantages such as deep-tissue penetration and high spatial resolution. Traditional NIR-II probes such as organic fluorophores usually suffer from poor photostability and potential long-term toxicity. Herein, we report the controlled synthesis of monodisperse NaCeF₄:Er/Yb nanocrystals (NCs) that exhibit intense NIR-II emission upon excitation at 980 nm. Ce³⁺ in the host lattice was found to enhance the luminescence of Er³⁺ at 1530 nm with a maximum NIR-II quantum yield of 32.8%, which is the highest among Er³⁺-activated nanoprobes. Particularly, by utilizing the intense NIR-II emission of NaCeF₄:Er/Yb NCs, we demonstrated their application as sensitive homogeneous bioprobes to detect uric acid with the limit of detection down to 25.6 nM. Furthermore, the probe was detectable in tissues at depths of up to 10 mm, which enabled in vivo imaging of mouse organs and hindlimbs with high resolution, thus revealing the great potential of these NaCeF₄:Er/Yb nanoprobes in deep-tissue diagnosis.

In the past few years, continuous efforts have been dedicated to developing NIR-II probes including organic fluorophores, carbon nanotubes, and semiconductor quantum dots (QDs). However, the use of these bioprobes has several limitations. For example, organic fluorophores commonly possess poor photostability and are susceptible to photobleaching. The applicability of QDs is compromised by photoblinking and high toxicity of heavy metal elements (e.g., cadmium and selenium). Moreover, both organic fluorophores and QDs may induce high background noise owing to a small Stokes shift, which decreases the detection sensitivity for bioassays. These concerns fuel high demand for a new generation of luminescent probes to circumvent the limitations of traditional ones.

Lanthanide (Ln³⁺)-doped nanocrystals (NCs), as another kind of promising luminescent probe, have received growing attention due to their tunable emissions from different Ln³⁺ activators. Compared with organic fluorophores and QDs, Ln³⁺-doped NCs feature long luminescence lifetime, high photostability, low toxicity and sharp f-f emission peaks. Thus, they are widely applied for in vitro bioassays and in vivo bioimaging. Nevertheless, most previous research studies focused on the exploration of UC nanoprobes with emission light in the UV or visible range, which may restrict the tissue penetration depth. Several Ln³⁺ ion (e.g., Pr³⁺, Nd³⁺, Sm³⁺, Dy³⁺, Ho³⁺, Er³⁺, Tm³⁺ and Yb³⁺) doped NCs have been reported to emit NIR-II light. However, the NIR-II quantum yields for most of these Ln³⁺-based NCs are still low for practical application. To meet the requirement of sensitive bioassays, it is urgent to develop...
Ln³⁺-doped NCs with highly efficient emission in the NIR-II region.

In this regard, we herein report the synthesis of monodispersive and size controllable Er³⁺/Yb³⁺-doped hexagonal NaCeF₄ core-only and core/shell NCs that exhibit intense NIR-II emission upon 980 nm excitation, by virtue of the efficient Yb³⁺–Er³⁺–Ce³⁺ energy transfer. The maximum NIR-II quantum yield for the NaCeF₄:Er/Yb NCs is determined to be 32.8%, which is ~17.5 times higher than that of the widely reported NaYF₄:Er/Yb NCs. Moreover, the tissue penetration depth of NIR-II emission from the proposed probe is found to be higher than that of the green UC emission of NaYF₄:Er/Yb NCs of similar particle sizes under otherwise identical conditions. After tail vein injection of hydrophilic NaCeF₄:Er/Yb NCs into nude mice, the biodistribution of the nanoprobes is clearly monitored for 24 h using an in vivo bioimaging system (Scheme 1).

Results and discussion

Hydrophobic and monodisperse NaCeF₄:Er/Yb NCs were synthesized via a facile high-temperature co-precipitation method. The X-ray diffraction (XRD) patterns of the as-prepared NCs can be indexed to pure hexagonal NaCeF₄ (JCPDS no. 75-1924), and no traces of other phases or impurities were detected (ESI Fig. S1†). Energy-dispersive X-ray (EDX) spectroscopy confirms the successful doping of Er³⁺/Yb³⁺ ions into the NaCeF₄ host (ESI Fig. S1†). By changing the reaction time at 320 °C, the sizes and morphologies of these NCs can be finely tailored. Specifically, longer reaction time resulted in larger particles. As shown in Fig. 1, when the reaction time increased from 20 to 30 min, the size of the obtained NaCeF₄:Er/Yb NCs increased markedly from 7.1 ± 0.5 to 25.2 ± 2.7 nm (Fig. 1a–c). With further increasing the reaction time to 45 min, the coexistence of small nanospheres and large nanorods was observed (Fig. 1d), which may be attributed to an Ostwald-ripening process where small particles dissolved and big nanorods grew simultaneously. After heating for 60 or 90 min, the small nanospheres completely transformed into larger nanorods with lengths of 103.3 ± 10.9 and 206.6 ± 16.5 nm (Fig. 1e–f), respectively.

Besides the core-only NCs, core/shell NCs were also synthesized through epitaxial growth of inert NaCeF₄ shells on the core-only NCs (7.1 ± 0.5 nm). These core/shell NCs, with an average size of 18.1 ± 1.9 nm, can be well dispersed in nonpolar organic solvents such as cyclohexane to form a stable transparent colloidal solution (ESI Fig. S2†). The high-resolution TEM (HRTEM) image shows a clearly observed d-spacing of 0.308 nm, which is in good agreement with the lattice spacing in the {0111} planes of hexagonal NaCeF₄, indicative of the high crystallinity of the as-prepared NCs.

Currently, Er³⁺/Yb³⁺-doped fluorides (e.g., NaYF₄) with low phonon energy are frequently reported as UC materials. For the typical UC emission process, the Yb³⁺ ion is usually used as the sensitizer to harvest 980 nm photons. An Er³⁺ ion is then excited to its excited states via two or more successive energy transfers from Yb³⁺ ions in close proximity, followed by radiative relaxation, resulting in UC emission of a higher-energy photon (Fig. 2a). Nevertheless, the energy gap between the F2/2 and F7/2 levels of Ce³⁺ (~2300 cm⁻¹) is close to that of the 4I15/2–4I13/2 energy gap (~3700 cm⁻¹) of Er³⁺. Therefore, for NaCeF₄:Er/Yb NCs, the 4I13/2 level of Er³⁺ is significantly populated through the efficient phonon-assisted nonradiative relaxation from the 4I15/2 level facilitated by Ce³⁺ ions. Upon excitation at 980 nm, intense DS emissions centered at ~1530 nm that are ascribed to the 4I15/2 → 4I13/2 transition of Er³⁺ were detected for all the synthesized Er³⁺/Yb³⁺-co-doped NaCeF₄ NCs (ESI Fig. S3 and S4†). With the size increasing from 7.1 nm to 200.6 nm, the NIR-II emission intensity increased by 4.1 times, and the effective PL lifetime of 4I13/2 was found to increase from 1.53 to 5.60 ms (ESI Fig. S5†).

The NIR-II absolute quantum yield (QY), defined as the ratio of the number of emitted photons to the number of absorbed photons, was determined to be as high as 32.8% for NaCeF₄:Er/
H₂O₂ or H₂O₂-generated biomolecules (Fig. 3a). In order to explore NCs can be explored as an effective bioprobe for the detection of H₂O₂ or H₂O₂-generated biomolecules (Fig. 3a). In order to investigate the quenching effect of H₂O₂ on the emission of NaCeF₄:Er/Yb NCs, the spectral response of ligand-free NaCeF₄:Er/Yb NCs with a size of 200.6 nm upon excitation by a 980 nm laser with a power density of ~100 W cm⁻² (ESI Fig. S3†). Meanwhile, impressive 3.6-fold and 13.6-fold enhancements of NIR-II emission at ~1530 nm for NaCeF₄:Er/Yb core-only and NaCeF₄:Er/Yb@NaCeF₄ core/shell NCs were observed relative to that of NaYF₄:Er/Yb NCs (20.1 ± 1.8 nm, ESI Fig. S6†), upon excitation at 980 nm (Fig. 2b). In sharp contrast, the visible UC emissions for Er³⁺ were negligibly weak in NaCeF₄:Er/Yb NCs, due to the effective depopulation of ⁴S₅/₂ and ⁵F₉/₂ levels in the presence of Ce³⁺ ions (Fig. 2c). The NIR-II absolute QYs were determined to be 1.9%, 5.6% and 19.5% for NaYF₄:Er/Yb, NaCeF₄:Er/Yb core-only, and NaCeF₄:Er/Yb@NaCeF₄ core/shell NCs, respectively.

To make the NaCeF₄:Er/Yb NCs hydrophilic for bioapplications, we removed the surface ligands through an acid treatment. Since Ce³⁺ ions in the host matrix were exposed on the surface of ligand-free NCs, which endows these NCs with excellent dispersibility in aqueous solutions. The successful synthesis of ligand-free NaCeF₄:Er/Yb NCs was verified by TGA, FTIR spectra and zeta-potential analyses (ESI Fig. S7–S9†). More importantly, the ligand-free NCs preserved the intense NIR-II emission from the OA-capped NCs with essentially unchanged intensity. The ζ potential of ligand-free NCs in aqueous solution was measured to be 21.9 ± 0.9 mV (ESI Fig. S9†) due to the existence of positively charged Ln³⁺ ions (i.e., Er³⁺, Yb³⁺ and Ce³⁺) on the surface of ligand-free NCs, which endows these NCs with excellent dispersibility in aqueous solutions.

Since Ce³⁺ ions in the host matrix were exposed on the surface of ligand-free NCs after the acid treatment, H₂O₂ can directly oxidize Ce³⁺ to Ce⁴⁺ through redox reaction, resulting in the quenching of NIR-II emission of Er³⁺ upon 980 nm excitation. Benefiting from such a redox reaction, NaCeF₄:Er/Yb NCs can be explored as an effective bioprobe for the detection of H₂O₂ or H₂O₂-generated biomolecules (Fig. 3a). In order to investigate the quenching effect of H₂O₂ on the emission of NaCeF₄:Er/Yb NCs, the spectral response of ligand-free NaCeF₄:Er/Yb NCs with a size of 25.2 ± 2.7 nm (0.5 mg mL⁻¹) upon addition of different amounts of H₂O₂ (0–10 μM) was measured upon 980 nm excitation (Fig. 3b). The integrated DSL intensity of NaCeF₄:Er/Yb decreased gradually with increasing concentration of H₂O₂, due to the redox reaction between the H₂O₂ and Ce⁴⁺ ions. As a result, the concentration of H₂O₂ can be quantified by the NIR-II emission intensity of NaCeF₄:Er/Yb NCs (Fig. 3c). In the control experiment, by utilizing NaYF₄:Er/Yb or NaYF₄:Er/Yb/Ce (with a Ce³⁺ content of 10 mol%) as the probe, a negligible photoluminescence (PL) quenching effect of Er³⁺ was observed upon addition of different concentrations of H₂O₂ (ESI Fig. S10†). The LOD, defined as the concentration that corresponds to 3 times the standard deviation above the signal measured in the blank, was determined to be 41.8 nM based on NaCeF₄:Er/Yb nanoprobes.

The highly sensitive response of H₂O₂ allows for the detection of biomarkers such as UA which can yield H₂O₂ through the UA/uricase reaction (Fig. 4a). The level of UA, which is the end product of purine metabolism in the human body in human blood and urine, can be treated as an indicator for certain clinical criteria. Abnormal levels of UA may cause diseases like gout, arthritis, renal disorder, Lesch–Nyhan syndrome, etc., specifically, excess UA in human blood is a risk factor in cardiovascular related diseases, while reduced UA levels (hypouricemia) have been found to be closely related to several diseases such as diabetes mellitus and AIDS. Therefore, the accurate detection of UA is of great importance in physiological survey and clinical diagnosis.

In the assay system, UA or uricase alone was not able to quench the NIR-II emission of NaCeF₄:Er/Yb nanoprobes upon 980 nm excitation, since no H₂O₂ was generated (Fig. 4b). However, a notable quenching in Er³⁺ emission was observed with the addition of both UA and uricase in NaCeF₄:Er/Yb.
solution. Meanwhile, it was found that a time of 3 h was needed to reach equilibrium for the NIR-II emission of Er3+ (ESI Fig. S11†). Under the optimized conditions (0.5 mg mL−1 NaCeF4:Er/Yb and 0.011 U mL−1 uricase), the integrated NIR-II emission intensity of Er3+ decreased gradually with UA concentration from 0 to 900 μM (Fig. 4c), due to the gradual release of H2O2. The calibration curve for the UA concentration exhibits a linear dependence in the range of 0.411–900 μM. The LOD of UA assay was determined to be 25.6 nM, which is much lower than the UA level in the serum of healthy human beings (130–460 μM). In order to verify the specificity of the bioassay, we performed control experiments by replacing UA with other possible interfering biomolecules and electrolytes that may exist in serum samples, such as metal ions, proteins, and amino acids, under otherwise identical conditions. As displayed in Fig. 5a, the quenching of NIR-II emission of Er3+ in the control groups was negligibly small, which is in marked contrast to the significant quenching effect caused by the addition of UA. Such an exclusive PL quenching in the experiment group confirms the high specificity of the assay, thus validating the applicability of NaCeF4:Er/Yb nanoprobes for UA detection in complex biological matrices such as serum.

For the detection of UA in human serum samples, the NIR-II signal of the serum-based detection system exhibited a linear dependence on the UA concentration ranging from 1.234 to 900 μM (ESI Fig. S12†). To show the reliability of direct quantitation of UA in complex biological fluids by applying the NaCeF4:Er/Yb nanoprobes, we carried out in vitro detection of UA in 24 serum samples. The UA concentrations determined by NaCeF4:Er/Yb nanoprobes were compared with those detected based on a commercial kit. As shown in Fig. 5b and Table S1, the UA levels determined from the NaCeF4:Er/Yb based assay are highly consistent with those from the commercial assay kit. The correlation coefficient between both kinds of assays was determined to be 0.98, demonstrating that the NC-based assay is as reliable as that using the commercial kit.

Moreover, we determined the recovery of three human serum samples upon addition of UA standard solutions with different concentrations. The analytical recoveries are in the range of 93.4–108.8% (Table 1). Both the coefficients of variation (CV) and recovery are within the acceptance criteria (CVs ≤ 15%, and recoveries in the range of 90–110%) set for bioanalytical method validation. These results clearly prove that the NaCeF4:Er/Yb nanoprobe has high reliability and practicability for UA

![Chemical equation](a) UA + Uricase + H2O + O2 → Allantoin + H2O2 + CO2

![Emission spectra](b) NIR-II emission spectra of 100 μL of NaCeF4:Er/Yb NCs (0.5 mg mL−1) after the addition of 100 μL of H2O, uricase (0.011 U mL−1), UA (900 μM) and uricase (0.011 U mL−1) + UA (900 μM), respectively, upon excitation at 980 nm. (c) Calibration curve for the UA assay. The control experiment was carried out by replacing UA with H2O under otherwise identical conditions.

![Calibration curve](a) Calibration curve for the UA concentration range from 0 to 900 μM. The LOQ of UA assay was determined to be 25.6 nM, which is much lower than the UA level in the serum of healthy human beings (130–460 μM). In order to verify the specificity of the bioassay, we performed control experiments by replacing UA with other possible interfering biomolecules and electrolytes that may exist in serum samples, such as metal ions, proteins, and amino acids, under otherwise identical conditions. As displayed in Fig. 5a, the quenching of NIR-II emission of Er3+ in the control groups was negligibly small, which is in marked contrast to the significant quenching effect caused by the addition of UA. Such an exclusive PL quenching in the experiment group confirms the high specificity of the assay, thus validating the applicability of NaCeF4:Er/Yb nanoprobes for UA detection in complex biological matrices such as serum.

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<table>
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<th>Added (μM)</th>
<th>Found (μM)</th>
<th>CV (%) n = 4</th>
<th>Recovery (%)</th>
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<td>5.1</td>
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</tr>
<tr>
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<tr>
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<td>—</td>
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<tr>
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<td>7.6</td>
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<tr>
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<td>—</td>
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detection in complex biological samples. Therefore, the proposed NaCeF₄:Er/Yb nanoprobe, exhibiting background-free NIR-II emission under NIR excitation, is highly desired as a homogeneous bioassay nanoplatform for accurate detection of UA and other H₂O₂-generated biomarkers in clinical bioassays. Compared to previously reported UA bioassay systems, the homogeneous assay carried out employing the NaCeF₄:Er/Yb nanoprobe is much more convenient and cost-effective, given that the assay can be performed based on a simple mixing of the test samples with uricase and the ligand-free NaCeF₄:Er/Yb nanoprobe, and no complicated operations are involved in either nanoprobe preparation or surface modification.

Another important application of NIR-II emission is the deep-tissue bioimaging. To make the as-prepared hydrophobic NCs biocompatible, we coated the surface of OA-capped NaCeF₄:Er/Yb@NaCeF₄ NCs with amphiphilic 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG₂₀₀₀-COOH) phospholipids (Lipo). The resultant Lipo-modified NaCeF₄:Er/Yb@NaCeF₄ NCs with a hydrodynamic diameter of 22.3 ± 1.1 nm were monodisperse in water (ESI Fig. S9†). As a proof-of-concept experiment to examine the tissue penetration ability of NIR-II emission, we covered Lipo-modified NaCeF₄:Er/Yb@NaCeF₄ and NaYF₄:Er/Yb NCs with pork muscle tissue of various thicknesses, which were imaged using a modified Maestro imaging system. As shown in Fig. 6a and b, the NIR-II luminescence of NaCeF₄:Er/Yb@NaCeF₄ NCs was detectable even at a depth of 10 mm upon excitation at 980 nm. By contrast, the green UC luminescence of NaYF₄:Er/Yb NCs can only be observed at 4 mm beneath the tissue surface under otherwise identical conditions. The penetration depths that correspond to 50% of the original signal of NIR-II and green luminescence were determined to be ~7 and ~3 mm, respectively. The higher depth of penetration of NIR-II emission is due to reduced tissue scattering of light within the NIR-II window compared with that in the visible range.

Furthermore, to demonstrate their great capability for noninvasive imaging, in vivo bioimaging experiments were carried out based on the Lipo-modified NaCeF₄:Er/Yb@NaCeF₄ and NaYF₄:Er/Yb nanoprobes via tail vein injection into mice with the same dosage (0.1 mg mL⁻¹, 1 mL). After 30 min of blood circulation, images were taken upon excitation at 980 nm with appropriately equipped filters. Fig. 6c shows the evolution of the PL signal over 24 h arising from the injection of NaCeF₄:Er/Yb@NaCeF₄ nanoprobes. 0.5 h after injection, the NCs accumulated essentially in the hindlimbs, liver, spleen, and lungs, as can be monitored by the bright NIR-II signals of Er³⁺. Particularly, images of the mouse blood vessels of organs and hindlimbs can be clearly observed, which reveals the excellent spatial resolution of NIR-II bioimaging. After longer time periods of blood circulation, PL fading from hindlimbs was observed. 24 h later, all the NaCeF₄:Er/Yb@NaCeF₄ nanoprobes accumulated in the liver. Note that no tissue autofluorescence signal and light scattering were detected.
such H2O2-responsive luminescence, we have achieved an LOD of emission of NaCeF4:Er/Yb@NaCeF4 in comparison with the UC dlimbs have been demonstrated by employing the distinct NIR-II spatial resolution in

More importantly, a deep tissue penetration depth and superior highly consistent with those measured independently using NIR-II QY for NaCeF4:Er/Yb NCs has been determined to be

intense NIR-II emissions at 1530 nm were realized because of

significant of NaCeF4:Er/Yb nanoprobes in practical applications.

Conclusions

In summary, we have developed a highly efficient NIR-II nanoprobe based on NaCeF4:Er/Yb NCs. Upon 980 nm excitation, intense NIR-II emissions at 1530 nm were realized because of efficient Yb3+-Er3+-Ce3+ energy transfer. The maximum absolute NIR-II QY for NaCeF4:Er/Yb NCs has been determined to be 32.8%, which is the highest among Er3+-activated NIR-II nanoprobes. Significantly, the NIR-II emission can be effectively inhibited by H2O2 produced via the UA/uricase reaction, due to the redox reaction between the H2O2 and Ce3+ ions. By virtue of such H2O2-responsive luminescence, we have achieved an LOD of 25.6 nM for UA detection. The concentrations of UA in 24 human serum samples determined using NaCeF4:Er/Yb nanoprobes were highly consistent with those measured independently using a commercial kit, showing the assay’s accuracy and reliability. More importantly, a deep tissue penetration depth and superior spatial resolution in in vivo imaging of mouse organs and hindlimbs have been demonstrated by employing the distinct NIR-II emission of NaCeF4:Er/Yb@NaCeF4 in comparison with the UC emission of NaF:Er/Yb. These findings reveal the great potential of NaCeF4:Er/Yb nanoprobes in practical in vivo detection of disease markers, which may open up a new route to the exploitation of Ln3+-doped NIR-II nanoprobes in versatile biomedical applications.

Experimental

Detailed experimental procedures are reported in the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references