



# Lanthanide-doped near-infrared II luminescent nanoprobe for bioapplications

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**ABSTRACT** Luminescent biosensing in the second near-infrared (NIR-II) region is featured with superior spatial resolution and high penetration depth by virtue of the suppressed scattering of long-wavelength photons. Hitherto, the reported NIR-II nanoprobe are mostly based on carbon nanotubes, organic fluorophores or semiconducting quantum dots. As an alternative, trivalent lanthanide ions (Ln<sup>3+</sup>) doped nanoparticles have been emerging as a novel class of promising nanoprobe. In this review, we highlight the recent progress in the design of highly efficient Ln<sup>3+</sup>-doped NIR-II nanoparticles towards their emerging bioapplications, with an emphasis on autofluorescence-free bioimaging, sensitive bioassay, and accurate temperature sensing. Moreover, some efforts and challenges towards this rapidly expanding field are envisioned.

**Keywords:** lanthanide ions, nanoprobe, near-infrared II luminescence, bioimaging, bioassay, temperature sensing

## INTRODUCTION

Luminescent probes play a crucial role in numerous bioapplications including bioimaging, biodetection, as well as disease diagnosis and therapeutics [1–10]. Currently, the visible emitting probes are widely used for *in vitro* studies. Nevertheless, their applications *in vivo* are limited by the strong absorption and scattering of visible lights in the biological media [11–13]. To circumvent these restrictions, luminescent probes exhibiting emission between 1,000 and 1,700 nm within the second near-infrared (NIR-II) region have been emerging in recent years since they can dramatically reduce scattering lights and increase penetration depth in biological applications, compared with those emitting in the visible or the first

NIR (NIR-I, 750–1,000 nm) regions [14–31].

To date, many advances have been made regarding the controlled synthesis, surface modification and optical properties of the NIR-II probes such as carbon nanotubes (CNTs) [32–36], organic fluorophores [37–39], semiconducting quantum dots (QDs) (e.g., PbSe and Ag<sub>2</sub>S) [40–42] and conjugated polymers [43]. However, CNTs usually exhibit broad emission bands (>300 nm) and low quantum yields (QY) (0.1–0.4%), which impedes their practical applications. For organic fluorophores, they may suffer from photobleaching and poor photostability. Besides, there are some unavoidable drawbacks for QDs such as photoblinking or intrinsic toxicity from the heavy metal elements (e.g., Pb and Cd). Conjugated polymers generally exhibit low solubility in aqueous solution. To these regards, it is of urgent demand to search for novel NIR-II luminescent nanoprobe to overcome the inherent limitations of the traditional ones.

Trivalent lanthanide ions (Ln<sup>3+</sup>) have the electron configuration of 4f<sup>n</sup>5s<sup>2</sup>5p<sup>6</sup> ( $n=1-13$ ). Due to the rich energy levels of Ln<sup>3+</sup>, their emissions cover the spectrum region from ultraviolet, visible to NIR. As an alternative to the traditional NIR-II probes, Ln<sup>3+</sup>-doped nanoparticles (NPs) are particularly intriguing owing to their superior properties, including high stability against photobleaching, long-lived ( $\mu\text{s}$ – $\text{ms}$ ) luminescence for time-gated detection, and narrow emission bands for multiplexed sensing [31,44–55]. All these features enable Ln<sup>3+</sup>-doped NIR-II NPs as an essential class of nanoprobe for diverse bioapplications. Several Ln<sup>3+</sup> ions (e.g., Yb<sup>3+</sup>, Tm<sup>3+</sup>, Er<sup>3+</sup>, Ho<sup>3+</sup>, Dy<sup>3+</sup>, Sm<sup>3+</sup>, Nd<sup>3+</sup> and Pr<sup>3+</sup>) were reported to produce NIR-II light (Fig. 1), but the NIR-II quantum yields (QYs) of most Ln<sup>3+</sup>-doped NPs were still

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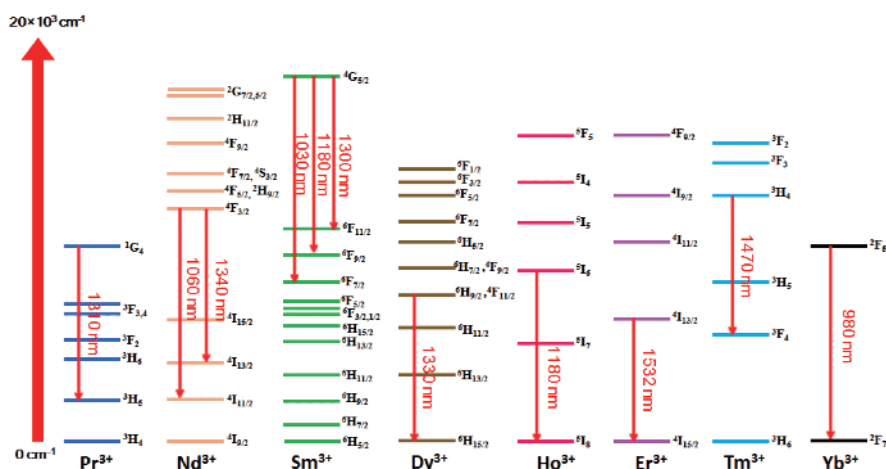


Figure 1 Energy level diagrams of Ln<sup>3+</sup> ions with typical emissions within the NIR-II region.

too low to fulfill their practical application in luminescent biosensing. As such, continuous efforts were dedicated to developing highly efficient Ln<sup>3+</sup>-doped NIR-II luminescent nano-bioprobes.

Although several classes of NIR-II luminescent probes like organic fluorophores, CNTs and QDs have already been well summarized in academic journals or books [58–61], a review focusing on Ln<sup>3+</sup>-based NIR-II nanoparticles is highly desired so far. Rather than being exhaustive, this review aims to present a comprehensive investigation about the most recent achievements in Ln<sup>3+</sup>-doped nano-bioprobes, which mainly covers from the strategies for improving the photoluminescence (PL) efficiency to their promising applications (Fig. 2). We start by the design strategy of the highly efficient NIR-II NPs, with emphasis on host selection, cation incorporation and surface modification. We then highlight their key bioapplications such as bioimaging, bioassay and temperature sensing, respectively. Finally, emerging trends and further efforts are proposed.

### DESIGN OF HIGHLY EFFICIENT NIR-II NANOPROBES

One of the bottlenecks for practical applications of NIR-II luminescent probes lies in their low QYs, which is defined as the ratio of the number of emitted photons to that of the absorbed photons. QYs of typical Ln<sup>3+</sup>-doped NIR-II NPs are summarized in Table 1, which indicates that most of them show low PL efficiency. In order to achieve high NIR-II luminescence output for commercial applications, researchers proposed several strategies such as host selection, cation incorporation, and surface mod-

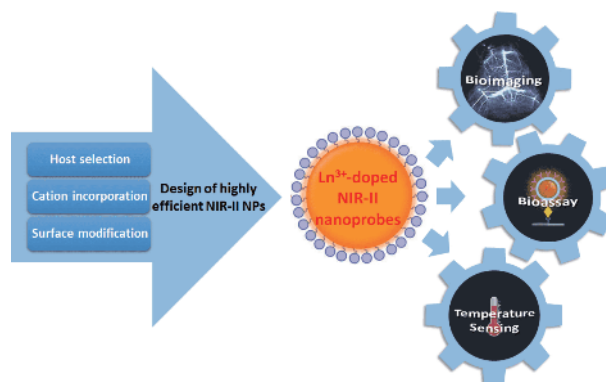


Figure 2 Overview of Ln<sup>3+</sup>-doped NIR-II nanoparticles from design strategies to bioapplications.

ification.

#### Host selection

The PL efficiency of Ln<sup>3+</sup> relies critically on the structure, crystal fields, local site symmetry and phonon energy of the host materials [45,50]. Thus, the selection of hosts determines the optical properties of the Ln<sup>3+</sup> dopants. Generally, desirable host materials should possess high optical damage threshold, low phonon energy, and close lattice matches to Ln<sup>3+</sup> dopants. Among these features, the phonon energy is a key parameter influencing their QYs. The host matrix with low energy phonons may suppress the multi-phonon relaxation process and reduce the non-radiative energy losses. This is important for Ln<sup>3+</sup> ions emitting in the NIR-II region, since the NIR-II emissions are easily quenched by high-energy vibrations [65]. Therefore, halide hosts (e.g., LiYF<sub>4</sub> [8], CaF<sub>2</sub> [63],

**Table 1** Optical characteristics and QYs of typical Ln<sup>3+</sup>-doped NIR-II NPs

Nanoprobe	Size (nm)	Excitation (nm)	Emission (nm)	QY (%)	Reference
LiYF <sub>4</sub> :Nd	18×25	808	900/1050	28	[8]
NaYF <sub>4</sub> :Yb/Er	183×113	975	1525	0.2	[44]
NaGdF <sub>4</sub> :Nd/Yb/Tm	21	800	980/1060	1.06	[56]
NaCeF <sub>4</sub> :Er/Yb	200.6	980	1530	32.8	[57]
NaNdF <sub>4</sub> :Mn	4.5	808	1058	10	[62]
CaF <sub>2</sub> :Y/Nd	10–15	808	989/1056/1328	9.3	[63]
NaGdF <sub>4</sub> :Nd@NaGdF <sub>4</sub>	15	740	900/1050/1330	40	[64]
NaYF <sub>4</sub> :Yb/Er@NaLuF <sub>4</sub>	26.2	980	1522	14	[66]
NaYbF <sub>4</sub> :Er/Ce@NaYF <sub>4</sub>	18	980	1550	0.27–2.73	[67]
NaYF <sub>4</sub> :Yb/Nd@CaF <sub>2</sub>	13	800	980/1060	~11	[68]
NaYF <sub>4</sub> :Yb/Nd@CaF <sub>2</sub>	12	808	980	20.7	[69]
NaYF <sub>4</sub> :Yb/Er@NaYbF <sub>4</sub> @NaYF <sub>4</sub> :Nd@ICG	52	800	1000/1064/1530	13.2	[70]
NaYF <sub>4</sub> :Er@ICG	~17	808	1520	3.1	[72]
CsPbCl <sub>3</sub> :Yb	16	380	980	170	[75]
CsPbCl <sub>1.5</sub> Br <sub>1.5</sub> :Yb/Ce	6.9	365	980	119	[77]
InP@YF <sub>3</sub> :Yb@LuF <sub>3</sub>	10	440	980	0.5	[79]

NaYF<sub>4</sub> [44], NaGdF<sub>4</sub> [64], LaF<sub>3</sub> [71] and SrF<sub>2</sub> [28]) with high chemical stability and low phonon energies are often selected as host matrix for the doping of Ln<sup>3+</sup> ions to achieve bright NIR-II emissions.

Because of the parity-forbidden nature of f–f transitions of Ln<sup>3+</sup> ions, the direct excitation for most Ln<sup>3+</sup> is usually inefficient. To overcome this, the strategy of sensitization of Ln<sup>3+</sup> ions by hosts like semiconductor NPs was proposed to enhance the absorption of excitation light [73,74]. As a consequence, the NIR-II luminescence of Ln<sup>3+</sup> is expected to be greatly enhanced *via* an efficient energy transfer from the semiconductor host to the doped Ln<sup>3+</sup> ions. Chen and co-workers reported the efficiently host-sensitized NIR-II emissions of Nd<sup>3+</sup> or Er<sup>3+</sup> ions doped in a series of semiconductor NPs such as In<sub>2</sub>O<sub>3</sub> [76], SnO<sub>2</sub> [78], TiO<sub>2</sub> [80] and ZnO [81,82]. To exemplify this, Er<sup>3+</sup> ions were doped into the lattice of SnO<sub>2</sub> NPs, which gave rise to NIR-II emissions of Er<sup>3+</sup> at 1.55 μm upon excitation above the SnO<sub>2</sub> bandgap (Fig. 3a). By monitoring the emission of Er<sup>3+</sup>, the PL excitation spectrum was dominated by an intense and broad band centered at 300 nm that corresponds to the bandgap absorption of SnO<sub>2</sub> NPs (Fig. 3b). Meanwhile, the emission spectrum displayed sharp NIR emission lines originating from the <sup>4</sup>I<sub>13/2</sub>→<sup>4</sup>I<sub>15/2</sub> transition of Er<sup>3+</sup> ions upon excitation at 300 nm, verifying the efficient energy transfer from SnO<sub>2</sub> host to emitters (Er<sup>3+</sup> ions).

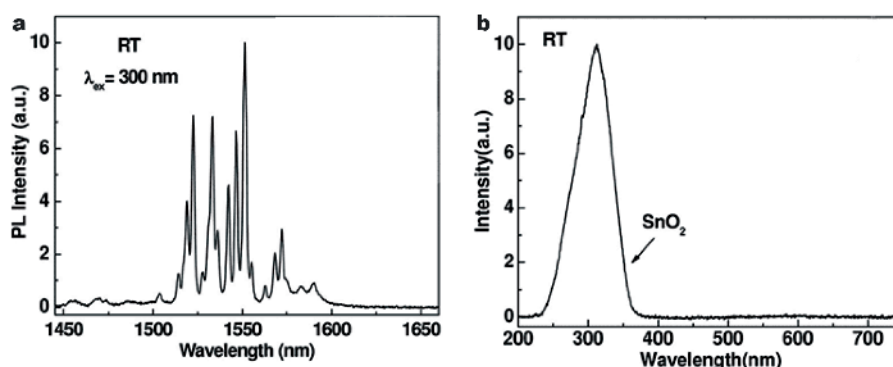
Recently, semiconducting QDs are also employed to

overcome the weak absorption of Ln<sup>3+</sup> ions for improving their PL efficiency, because QDs have strong broadband absorption and tunable bandgap. However, the synthesis of Ln<sup>3+</sup>-doped QDs remains a great challenge. In one of the earliest reports, Yb<sup>3+</sup> was doped in CdSe QDs through a three-step synthesis strategy [83]. However, only weak emission of Yb<sup>3+</sup> was achieved upon excitation above the CdSe bandgap at 580 nm. Due to the large absorption cross-section of perovskite QDs, much effort was recently devoted to introducing Ln<sup>3+</sup> ions into CsPbX<sub>3</sub> perovskite QDs (e.g., CsPbCl<sub>3</sub> [75], CsPbBr<sub>3</sub> [84,85], CsPbI<sub>3</sub> [85], CsPbCl<sub>1.5</sub>Br<sub>1.5</sub> [77,86]) in order to realize strong NIR emission of Yb<sup>3+</sup>. The NIR luminescent QY for CsPbCl<sub>3</sub>:Yb NPs was reported to be as high as 170% upon excitation at 380 nm [75]. In addition to Ln<sup>3+</sup>-doped QDs, core/shell structures were fabricated for the sensitization of Ln<sup>3+</sup> ions. Alivisatos and co-workers [79] designed InP/Ln<sub>x</sub>Y<sub>1-x</sub>F<sub>3</sub>/ShF<sub>3</sub> (Ln=Yb/Nd, Sh=Lu/Y) core/shell/shell NPs to realize the broad excitation of Yb<sup>3+</sup> *via* the sensitization of InP.

### Cation incorporation

Intentional cation incorporation is an effective way to manipulate the optical properties of Ln<sup>3+</sup>-doped NPs by either controlling the energy transfer process or altering the crystal field and the local site symmetry of Ln<sup>3+</sup> ions.

Some cations (e.g., Ln<sup>3+</sup> or transition metal ions) were usually chosen as “energy donor” ions by virtue of their



**Figure 3** (a) Emission spectrum of SnO<sub>2</sub>:Er NPs upon excitation at 300 nm. (b) Excitation spectrum of SnO<sub>2</sub>:Er NPs by monitoring the emission at 1,551.2 nm. Adapted with permission from Ref. [78]. Copyright 2009, Optical Society of America.

relatively large absorption cross section. For instance, Yb<sup>3+</sup> or Nd<sup>3+</sup> ions are widely doped as sensitizers to harvest the NIR excitation photons (980 or 808 nm) and transfer the energy to Ln<sup>3+</sup> emitters [46,73]. Er<sup>3+</sup> or Ho<sup>3+</sup> ions were also reported to serve as sensitizers to harvest the excitation lights of 1,532 and 1,150 nm, respectively [87–89]. For transition metal ions, Bi<sup>3+</sup> was co-doped with Yb<sup>3+</sup> in Gd<sub>2</sub>O<sub>3</sub> NPs to enhance the NIR-II emission of Yb<sup>3+</sup> *via* cooperative energy transfer under broad-band ultraviolet excitation (250–400 nm) of Bi<sup>3+</sup> [90].

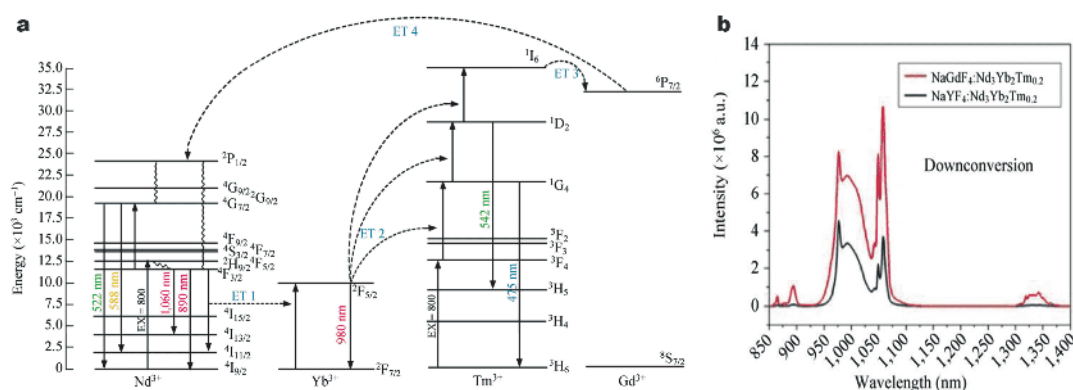
Besides sensitization, cation incorporation may also act as a bridge for energy transfer. The co-doping of Ce<sup>3+</sup> with Er<sup>3+</sup> and Yb<sup>3+</sup> ions enhanced the NIR-II emission of Er<sup>3+</sup> [57,67,91]. High QY of Er<sup>3+</sup> (i.e., 0.27–2.73%) was achieved for NaYbF<sub>4</sub>:Er/Ce@NaYF<sub>4</sub> core/shell NPs in aqueous solutions [67]. In these NPs, the upconversion (UC) pathway of Er<sup>3+</sup> was suppressed while the downshifting (DS) emission of Er<sup>3+</sup> at 1,550 nm was boosted by ~9 times. Similar phenomenon was observed by Chen and co-workers in NaCeF<sub>4</sub>:Er/Yb NPs [57]. The <sup>4</sup>I<sub>13/2</sub> state of Er<sup>3+</sup> can be markedly populated through the efficient phonon-assisted non-radiative relaxation from the <sup>4</sup>I<sub>11/2</sub> state facilitated by Ce<sup>3+</sup> ions, due to small energy mismatch between <sup>2</sup>F<sub>5/2</sub>→<sup>2</sup>F<sub>7/2</sub> of Ce<sup>3+</sup> (~2,300 cm<sup>-1</sup>) and <sup>4</sup>I<sub>11/2</sub>→<sup>4</sup>I<sub>13/2</sub> of Er<sup>3+</sup> (~3,700 cm<sup>-1</sup>). Gd<sup>3+</sup> was also reported to serve as the intermediate bridge ions to promote the energy transfer from the accumulator ions to Ln<sup>3+</sup> ions. For example, an efficient energy transfer pathway of Nd<sup>3+</sup> → Yb<sup>3+</sup> → Tm<sup>3+</sup> → Gd<sup>3+</sup> → Nd<sup>3+</sup> in NaGdF<sub>4</sub>:Nd/Yb/Tm NPs was proposed by Chaudhuri and co-workers [56]. In the NaGdF<sub>4</sub>:Nd/Yb/Tm NPs, Gd<sup>3+</sup> served as a bridge to transfer the energy from <sup>1</sup>I<sub>6</sub> of Tm<sup>3+</sup> to <sup>2</sup>P<sub>1/2</sub> of Nd<sup>3+</sup>, followed by a non-radiative relaxation to the <sup>4</sup>F<sub>3/2</sub> and <sup>4</sup>G<sub>7/2</sub> multiplets, resulting in the intense NIR-II emission of Nd<sup>3+</sup> (Fig. 4a). Significantly enhanced NIR-II emission

intensity of Nd<sup>3+</sup> by ~3 times was realized as compared with that of Gd<sup>3+</sup>-free counterparts (i.e., NaYF<sub>4</sub>:Nd/Yb/Tm NPs) under otherwise identical conditions (Fig. 4b).

Additionally, co-doping with Y<sup>3+</sup> ions was reported to enhance the NIR-II emission intensity of Nd<sup>3+</sup> by Quintanilla and co-workers [92]. In their work, the addition of Y<sup>3+</sup> ions into the CaF<sub>2</sub> host avoided clustering and PL quenching of Nd<sup>3+</sup> ions. Similarly, Zhang and co-workers adopted the same strategy to improve the PL efficiency of CaF<sub>2</sub>:Nd NPs by co-doping with Y<sup>3+</sup> [63]. As a result, a high NIR-II QY of 9.30% was achieved, which was ~3 times higher than that of CaF<sub>2</sub>:Nd NPs.

### Surface modification

The deleterious surface quenching effect in the colloidal dispersions deriving from their high surface area-to-volume ratio of NPs strongly affects the QY of Ln<sup>3+</sup>-doped NPs. To minimize the PL quenching of emitters from the surface ligands and liquid media, designing high-quality core/shell structure is frequently used [93,94]. For example, silica shell coating was adopted to suppress the vibrational quenching caused by the O–H groups [64,95]. As a result, the NIR-II emission intensity of Ln<sup>3+</sup> dopants in the core NPs can be increased. Moreover, it was found that surface passivation by growing a uniform shell with similar lattice parameters was more efficient to improve the NIR-II QY of Ln<sup>3+</sup>-doped NPs. For instance, the NIR-II emission intensity of NaGdF<sub>4</sub>:Nd NPs was increased by 1.82 times after coating with a 2-nm NaGdF<sub>4</sub> shell [64]. The NIR-II QY of Nd<sup>3+</sup> in these NaGdF<sub>4</sub>:Nd@NaGdF<sub>4</sub> core/shell NPs was as high as 40%. Likewise, the NIR-II emission intensity of NaYF<sub>4</sub>:Yb/Nd core NPs was enhanced by 45 times after coating with the CaF<sub>2</sub> shell [68]. Recently, Alivisatos and co-workers [66] investigated the

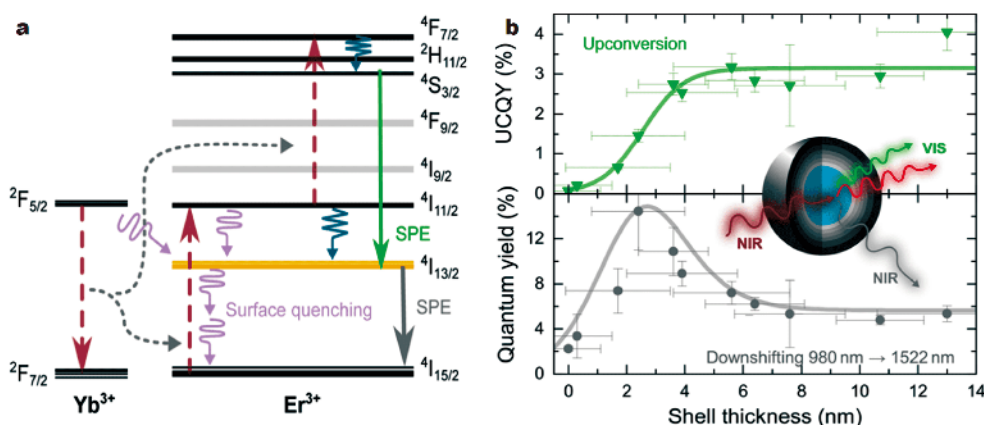


**Figure 4** (a) Scheme illustration of the energy transfer between  $\text{Nd}^{3+}$ ,  $\text{Yb}^{3+}$ ,  $\text{Tm}^{3+}$  and  $\text{Gd}^{3+}$  in  $\text{NaGdF}_4:\text{Nd}/\text{Yb}/\text{Tm}$  NPs. (b) NIR emission spectra of  $\text{NaGdF}_4:\text{Nd}/\text{Yb}/\text{Tm}$  and  $\text{NaYF}_4:\text{Nd}/\text{Yb}/\text{Tm}$ , respectively. Adapted with permission from Ref. [56]. Copyright 2014, Tsinghua University Press and Springer-Verlag GmbH Germany, part of Springer Nature.

relationship between shell thickness and the NIR-II emission intensity of  $\text{Er}^{3+}$  in  $\text{NaYF}_4:\text{Yb}/\text{Er}@/\text{NaYF}_4$  core/shell NPs. They demonstrated that surface quenching assisted downshifting (SQAD) processes increased the population of  $^4\text{I}_{13/2}$   $\text{Er}^{3+}$  energy level in thin shelled NPs due to energy transfer from  $^4\text{I}_{11/2}$  of  $\text{Er}^{3+}$  or  $^4\text{F}_{5/2}$  of  $\text{Yb}^{3+}$  to  $^4\text{I}_{13/2}$  of  $\text{Er}^{3+}$  (Fig. 5a). The NIR-II QY of  $\text{Er}^{3+}$  decreased markedly in  $\text{NaYF}_4:\text{Yb}/\text{Er}@/\text{NaYF}_4$  NPs with very thick shells where the SQAD processes were suppressed. Thus, as shown in Fig. 5b, there existed an optimized shell thickness (2.4 nm) for the highest NIR-II QY of  $\text{Er}^{3+}$ . In comparison with the NIR-II QY of  $\text{Er}^{3+}$ , the UC QY of  $\text{Er}^{3+}$  gradually increased and reached a stable plateau when the thickness of the shell was above  $\sim 5$  nm.

Besides coating with an inorganic shell, surface modification with organic molecules (e.g., tropolonate, indocyanine green) was also adopted for the efficient NIR-

II emission of  $\text{Ln}^{3+}$  [70,96]. As a typical example, indocyanine green (ICG) was coated on the surface of  $\text{NaYF}_4:\text{Yb}/\text{X}@/\text{NaYF}_4:\text{Nd}$  ( $\text{X} = \text{Er}, \text{Ho}, \text{Tm}, \text{or Pr}$ ) NPs to increase their NIR-II emission intensity [70]. In the ICG sensitized core-shell-shell (CSS) NPs, the energy transfer efficiency from ICG to the NPs was  $\sim 75\%$ . Meanwhile, their NIR-II QY was  $\sim 13\%$ . Note that the layer of organic molecule may passivate the surface of  $\text{Ln}^{3+}$ -doped NPs and endow them with water solubility when they are transferred from a hydrophobic environment to hydrophilic one [8,69,73]. Such a feature is particularly important for NIR-II emissions of  $\text{Ln}^{3+}$  which is sensitive to aqueous media due to small energy gap between their excited and ground states [45,65]. To this end, several ligands like poly(acrylic acid) [73,97], polyethyleneimine [44], poly(maleic anhydride-*alt*-1-octadecene)-polyethylene glycol (PMH-PEG) [67] were



**Figure 5** (a) Scheme illustration of the SQAD model in  $\text{NaYF}_4:\text{Yb}/\text{Er}@/\text{NaYF}_4$  core/shell NPs. (b) Shell thickness-dependent UC QY (green) and NIR-II QY (gray) of  $\text{Er}^{3+}$  in  $\text{NaYF}_4:\text{Yb}/\text{Er}@/\text{NaYF}_4$  core/shell NPs upon excitation at 980 nm. Adapted with permission from Ref. [66]. Copyright 2016, American Chemical Society.



modified on the surface of  $\text{Ln}^{3+}$ -doped NPs to improve their hydrophilicity.

## BIOIMAGING

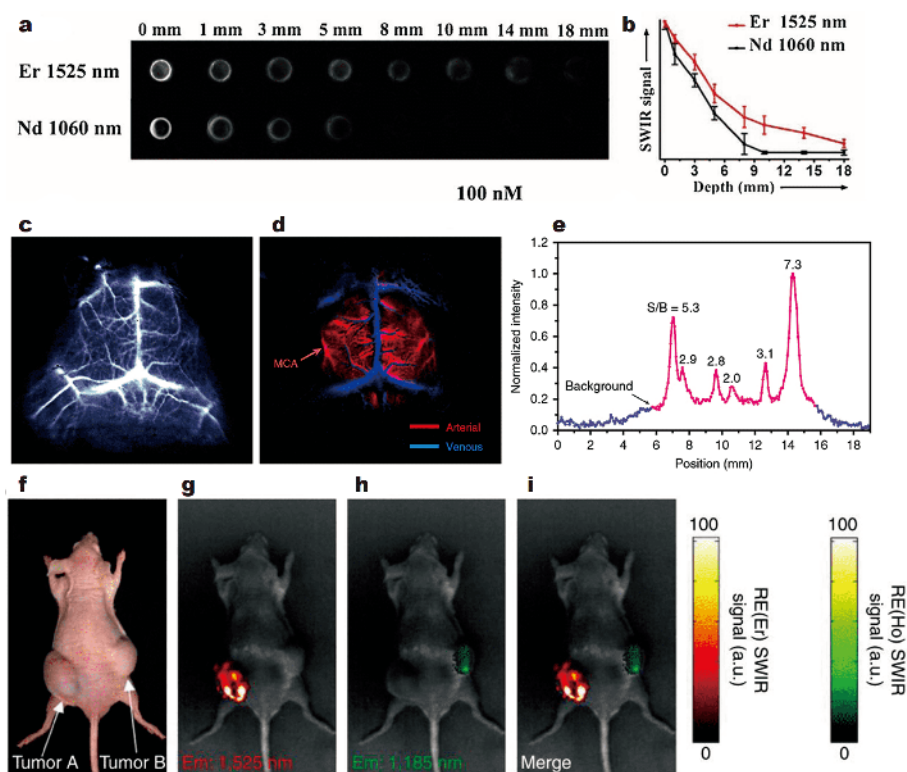
Bioimaging techniques are widely applied in clinical diagnosis and therapy. Among various bioimaging modalities, optical bioimaging attracts reviving interest due to its fast response, high sensitivity and superior resolution. However, there remains a challenge of conventional fluorescent probes for bioimaging, due to their inherent limitations like shallow tissue penetration and poor signal-to-noise ratio (SNR). In recent years,  $\text{Ln}^{3+}$ -doped NIR-II NPs have been considered as promising luminescent nanoprobes for bioimaging. The emission wavelength, excitation wavelength and PL lifetime can be exquisitely manipulated by changing the  $\text{Ln}^{3+}$  dopants, hosts and structure of nanomaterials, which renders them an ideal candidate for use in diverse bioimaging applications.

The NIR-II emissions of  $\text{Ln}^{3+}$ -doped NPs can be tuned to meet the requirements of the *in vitro* and *in vivo* bioimaging. In the past few years, several  $\text{Nd}^{3+}$ -doped NPs such as  $\text{NdF}_3@\text{SiO}_2$  [95],  $\text{CaTiO}_3:\text{Nd}$  [98],  $\text{NaGdF}_4:\text{Nd}@\text{NaGdF}_4$  [99],  $\text{LiLuF}_4:\text{Nd}@\text{LiLuF}_4$  [100] and  $\text{NaYF}_4:\text{Nd}@\text{NaGdF}_4$  [101] were extensively investigated as probes for bioimaging. Among them,  $\text{NdF}_3@\text{SiO}_2$  NPs exhibiting emission from  ${}^4\text{F}_{3/2} \rightarrow {}^4\text{I}_{11/2}$  of  $\text{Nd}^{3+}$  centered at  $\sim 1,060$  nm were first employed by Wang *et al.* [95] for bioimaging of living tissues in 2008. However, Villa *et al.* [28] pointed out that NPs emitting around 1,000 nm failed to completely eliminate the autofluorescence from the strong NIR fluorescence ( $\sim 1,100$  nm) generated by the mouse food upon excitation at 808 nm. To overcome this issue, they utilized the NIR-II emission from  ${}^4\text{F}_{3/2} \rightarrow {}^4\text{I}_{13/2}$  of  $\text{Nd}^{3+}$  centered at 1,340 nm from  $\text{SrF}_2:\text{Nd}$  NPs for deep-tissue and autofluorescence-free *in vivo* imaging. Moreover, Wang *et al.* [102] compared the penetration depth between emission at 1,060 and 1,525 nm in bioimaging. It was found that the emission light of 1,525 nm showed higher penetration depth than that of 1,060 nm (Fig. 6a, b). Subsequently, Diao *et al.* [23] also proposed that *in vivo* imaging based on emission beyond 1,500 nm afforded the high SNR and superior spatial resolution in bioimaging. The  $\text{NaYbF}_4:\text{Er}/\text{Ce}@\text{NaYF}_4$  NPs exhibiting 1,550 nm emission were employed for bioimaging of the blood vasculature in the hindlimb of mouse. The SNR of NIR-II bioimaging was determined to be 4.50, which is much higher than that of NIR-I bioimaging (i.e., 1.19). Meanwhile, a spatially resolved blood-flow map in the mouse brain can be

captured in a very short exposure time (20 ms) (Fig. 6c–e) [67]. Inspired by this work,  $\text{NaYF}_4:\text{Gd}/\text{Yb}/\text{Er}$  nanorods were applied for bioimaging of tiny tumor (4 mm in diameter), by virtue of the highly efficient NIR-II emission at 1,520 nm of  $\text{Er}^{3+}$  [73].

Besides the emission wavelength, the selection of excitation wavelength is also of vital importance. Nowadays, the majority of  $\text{Ln}^{3+}$ -doped NIR-II nanoprobes were excited at 980 nm, where  $\text{Yb}^{3+}$  ions were used as sensitizer. Typical examples include  $\text{NaYF}_4:\text{Yb}/\text{Er}$  [46],  $\text{NaCeF}_4:\text{Er}/\text{Yb}@\text{NaCeF}_4$  [57],  $\text{NaYF}_4:\text{Yb}/\text{Er}/\text{Mn}$  [103], to name a few. In one pioneering work, Naczynski *et al.* [46] demonstrated the application of  $\text{NaYF}_4:\text{Yb}/\text{Er}$  NPs for *in vivo* NIR-II bioimaging upon excitation at 980 nm (Fig. 6f–i). Later, it was found that the excitation light of 980 nm might not be the ideal choice for bioimaging since the strong optical absorption of water at 980 nm may cause undesired tissue heating effect [104]. In this regard, the excitation at 740–800 nm with lower water absorption in tissue was proposed. Thus,  $\text{Nd}^{3+}$  as a sensitizer to harvest  $\sim 800$  nm light received increasing attention [6,105]. For instance, Wang *et al.* [102] synthesized multi-shelled NPs based on  $\text{NaGdF}_4@\text{NaGdF}_4:\text{Yb}/\text{Er}@\text{NaYF}_4:\text{Yb}@\text{NaNdF}_4:\text{Yb}$  and demonstrated their great potential for bioimaging in deep tissues upon excitation at 800 nm. Likewise, Prasad *et al.* [64,70] designed  $\text{NaGdF}_4:\text{Nd}@\text{NaGdF}_4$  NPs which can be excited at 740 nm for bioimaging. Because tissue absorption and scattering are minimized in NIR-II spectral range, high penetration depth can be expected by tuning the excitation light from NIR-I to NIR-II region. In this regard, Liu *et al.* [87] presented a new type of  $\text{Er}^{3+}$ -sensitized NPs ( $\text{NaErF}_4:\text{Ho}@\text{NaYF}_4$ ) with the excitation (1,530 nm) and emission (1,180 nm) located in NIR-II region. Nevertheless, the excitation at 1,530 nm locates in the strong absorption band of water, which may generate some overheating problems [106]. As such, X-rays instead of NIR light was proposed by Naczynski *et al.* [86] as excitation light to produce NIR-II emission of  $\text{Er}^{3+}$  ions from  $\text{NaYF}_4:\text{Yb}/\text{Er}$  NPs for lymphatic mapping (Fig. 7). The use of X-rays may overcome the problem of the limited penetration depth of NIR excitation lights.

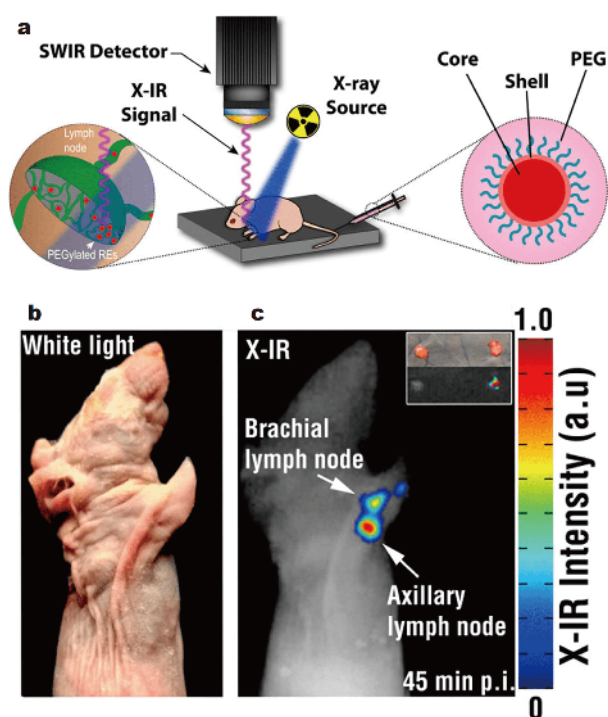
In addition to the tuning of emission and excitation wavelength, lifetime manipulation also plays an important role in bioimaging. Taking advantage of long PL lifetime of  $\text{Ln}^{3+}$ , del Rosal *et al.* [107] realized time-gated *in vivo* imaging by employing  $\text{NaGdF}_4:\text{Nd}$  NPs to remove the food-related infrared autofluorescence. When the delay time was set to be 1  $\mu\text{s}$ , short-lived background autofluorescence was no longer present while only long-



**Figure 6** (a) Comparison of the penetration depth of 1,532-nm emission from  $\text{Er}^{3+}$  and 1,060-nm emission from  $\text{Nd}^{3+}$ . (b) Signal intensity curve obtained from (a). Reprinted with permission from ref. [102]. Copyright 2014, Wiley-VCH Verlag GmbH & Co. KGaA. (c) Cerebral vascular image in NIR-II region with 20 ms exposure time and (d) corresponding principal component analysis of overlaid image showing arterial (red) and venous (blue) vessels. (e) Signal to background (SBR) analysis of cerebrovascular image in NIR-II region. Reprinted with permission from ref. [67]. Copyright 2017, Nature Publishing Group. (f) Bright field image of the tumor-bearing nude mouse. (g–i) NIR-II *in vivo* imaging after injecting  $\text{NaYF}_4:\text{Yb}/\text{Er}$  and  $\text{NaYF}_4:\text{Yb}/\text{Ho}$  NPs separately on left and right flank of the mouse. Reprinted with permission from ref. [46]. Copyright 2013, Nature Publishing Group.

lived emission of  $\text{Nd}^{3+}$  at 1,060 nm was detected. Owing to their tunability of PL lifetime in a wide range from  $\mu\text{s}$  to ms,  $\text{Ln}^{3+}$ -doped NIR-II NPs are also an ideal candidate for time-domain multiplexing bioimaging [108]. The strategy for manipulating the PL lifetime of  $\text{Ln}^{3+}$ -doped NIR-II NPs primarily involves the control of dopant combinations as well as the host structure [109,110]. Hitherto, only a few reports demonstrated the applicability of  $\text{Ln}^{3+}$ -doped NIR-II NPs for *in vivo* multiplexing lifetime imaging. Ortgies *et al.* [109] achieved *in vivo* NIR-II lifetime-based multiplexed imaging by utilizing two types of  $\text{NaYF}_4:\text{Yb},\text{Nd}@\text{CaF}_2$  NPs with different lifetimes (Fig. 8a). The one with shorter NIR-II PL lifetime was intruded in the mouse through oral administration and the other one with longer lifetime was intruded through intravenous injection. These two types of NPs can be distinguished from the lifetime imaging by a custom-made software. Correspondingly, their different biodistribution routes after oral or intravenous adminis-

tration were clearly tracked and visualized (Fig. 8b). By comparison, the biodistribution routes cannot be distinguished from intensity-based PL imaging (Fig. 8c). Furthermore, Fan *et al.* [110] performed *in vivo* multiplexed imaging by employing  $\text{Ln}^{3+}$ -doped NIR-II NPs with engineered PL lifetimes. They carried out cancer diagnostics *in vivo* with three kinds of core-shell  $\text{NaGdF}_4@\text{NaGdF}_4:\text{Yb}/\text{Er}@/\text{NaYF}_4:\text{Yb}@/\text{NaNdF}_4:\text{Yb}$  NPs (Er-NPs) of different lifetimes conjugated with primary antibodies, which were used for targeting three typical biomarkers of MCF-7 breast cancer cells (i.e., oestrogen receptor, progesterone receptor and human epidermal growth factor receptor-2), respectively. After injecting these NPs to nude mice bearing xenografted tumors, the biomarker expressions of the three markers can be identified by lifetime imaging. Their result showed excellent correlation with conventional immunohistochemical methods, indicating that lifetime imaging is a feasible approach for accurate quantification



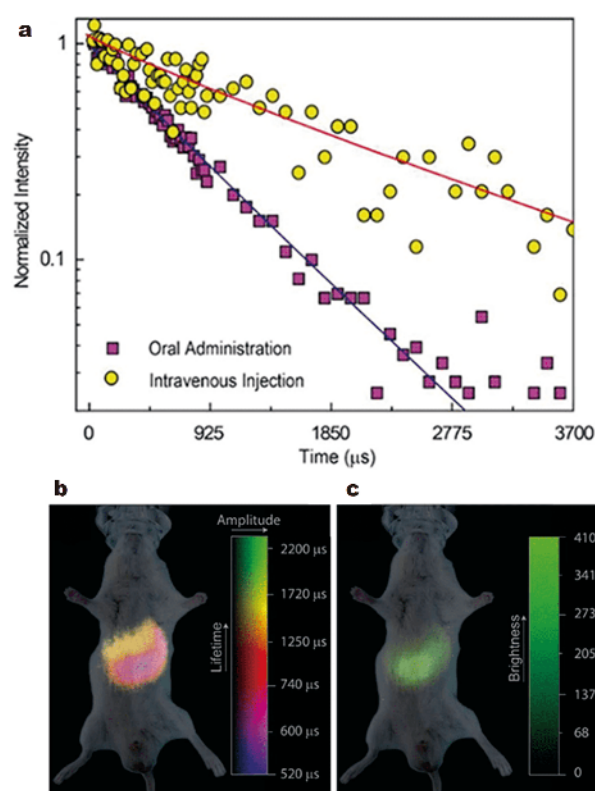
**Figure 7** (a) Schematic diagram of *in vivo* NIR-II imaging based on PEGylated  $\text{NaYF}_4:\text{Yb}/\text{Er}$  NPs upon excitation with X-ray light. (b) Bright field image of nude mouse and (c) NIR-II lymphatic mapping of the mouse injected with PEGylated  $\text{NaYF}_4:\text{Yb}/\text{Er}$  NPs upon excitation with X-ray light. Adapted with permission from Ref. [86]. Copyright 2015, American Chemical Society.

of disease markers.

## BIOASSAY

Luminescent bioassay is a powerful technique utilizing optical probes for detecting trace amount of target analytes, which is essential for many biomedical applications like pharmaceutical preparation, disease diagnosis and therapy. Currently, a variety of  $\text{Ln}^{3+}$ -doped NPs exhibiting visible emissions have been developed for bioassays [111–116]. However, the bioassays based on  $\text{Ln}^{3+}$ -doped NIR-II NPs have been rarely reported.

The  $\text{Ln}^{3+}$ -doped NIR-II NPs with high NIR-II QYs are favorable for sensitive bioassays. To meet the requirement of sensitive bioassay, Lei *et al.* [57] recently developed a novel NIR-II nanoprobe of  $\text{NaCeF}_4:\text{Er}/\text{Yb}$  NPs. Owing to the efficient  $\text{Yb}^{3+}-\text{Er}^{3+}-\text{Ce}^{3+}$  energy transfer, a maximum NIR-II QY of 32.8% for  $\text{NaCeF}_4:\text{Er}/\text{Yb}$  was achieved, which was higher than that of other  $\text{Er}^{3+}$ -activated nanoprobles. They found that the NIR-II emission of  $\text{Er}^{3+}$  in  $\text{NaCeF}_4:\text{Er}/\text{Yb}$  NPs can be effectively inhibited by  $\text{H}_2\text{O}_2$ , due to the redox reaction between the  $\text{Ce}^{3+}$  ions and  $\text{H}_2\text{O}_2$  (Fig. 9a). The limit of detection (LOD) for  $\text{H}_2\text{O}_2$  was

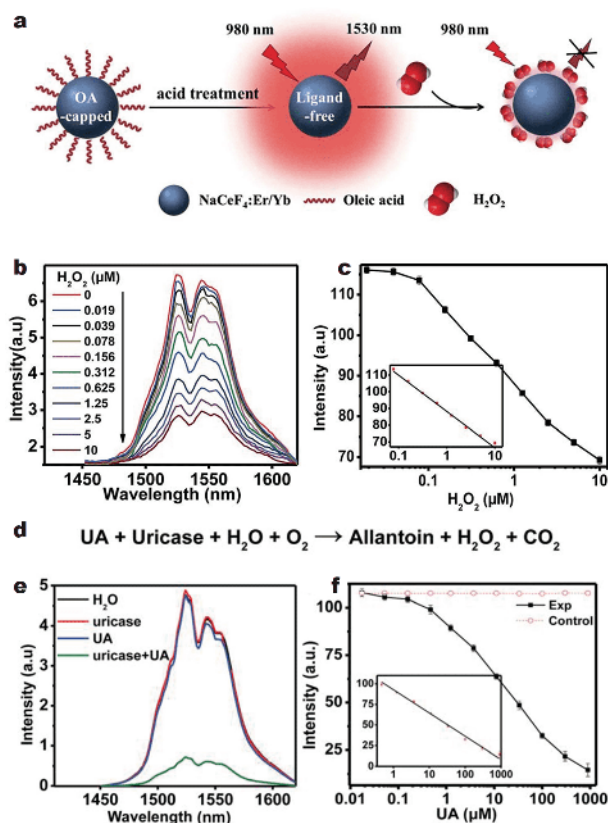


**Figure 8** (a) PL decays of two types of NPs ( $\text{NaY}_{0.9-x}\text{Yb}_{0.1}\text{Nd}_x\text{F}_4@\text{CaF}_2$ ,  $x=0.2$  and  $0.3$ , respectively). *In vivo* multiplexed imaging of mouse after oral and intravenous injection of these two types of NPs based on (b) PL lifetime and (c) PL intensity. Adapted with permission from Ref. [109]. Copyright 2018, American Chemical Society.

determined to be  $41.8 \text{ nmol L}^{-1}$  by using  $\text{NaCeF}_4:\text{Er}/\text{Yb}$  nanoprobles (Fig. 9b, c). Based on the  $\text{H}_2\text{O}_2$ -responsive luminescence, we also demonstrated their application as homogeneous nanoprobles to detect uric acid (UA), since  $\text{H}_2\text{O}_2$  can be produced through the UA/uricase reaction (Fig. 9d–f). Accordingly, an LOD of  $25.6 \text{ nmol L}^{-1}$  was achieved for UA. Moreover, the concentrations of UA in serum samples determined by the  $\text{NaCeF}_4:\text{Er}/\text{Yb}$  NPs were highly consistent with those measured by commercial kit, indicative of the assay's accuracy and reliability. These results revealed the great potential of  $\text{Ln}^{3+}$ -doped NIR-II NPs for practical *in vitro* detection of disease markers.

To show the superiority of  $\text{Ln}^{3+}$ -doped NIR-II NPs for *in vivo* bioassays, Zhang and co-workers [87] reported another NIR-II nanoprobe of  $\text{NaErF}_4:\text{Ho}@\text{NaYF}_4$  NPs with both excitation (1,530 nm) and emission (1,180 nm) located in the NIR-II region. For  $\text{NaErF}_4:\text{Ho}@\text{NaYF}_4$  NPs,  $\text{Er}^{3+}$  ions acted as both sensitizer and emitter for harvesting pump photons at 1,530 nm and subsequently





**Figure 9** (a) Schematic illustration of the procedure for homogeneous assay of H<sub>2</sub>O<sub>2</sub> with NaCeF<sub>4</sub>:Er/Yb NPs. (b) NIR-II emission intensity of ligand-free NaCeF<sub>4</sub>:Er/Yb NPs with different concentrations of H<sub>2</sub>O<sub>2</sub>, upon excitation at 980 nm. (c) Calibration curve for homogeneous assay of H<sub>2</sub>O<sub>2</sub>. Inset reveals the linear region of the calibration curve (0.078–10 μmol L<sup>-1</sup>). (d) Chemical equation for the generation of H<sub>2</sub>O<sub>2</sub> via UA/uricase reaction. (e) NIR-II emission spectra of NaCeF<sub>4</sub>:Er/Yb NPs after adding H<sub>2</sub>O, uricase, UA and uricase + UA, respectively, upon excitation at 980 nm. (f) Calibration curve of UA assay. Inset reveals the linear region of the calibration curve (0.411–900 μmol L<sup>-1</sup>). Adapted with permission from Ref. [57]. Copyright 2018, the Royal Society of Chemistry.

generating the UC emission at 980 nm. Meanwhile, Ho<sup>3+</sup> ions also served as an emitter to produce UC emission at 1,180 nm. By virtue of the multiple emissions, the proposed NIR-II NPs can be utilized as ratiometric fluorescent H<sub>2</sub>O<sub>2</sub> sensor. Specifically, NaErF<sub>4</sub>:Ho@NaYF<sub>4</sub> NPs and organic chromophore probe IR1061 with strong absorption at 800–1,100 nm were encapsulated in polycaprolactone. In the absence of H<sub>2</sub>O<sub>2</sub>, the 980 nm emission ( $I_{980}$ ) of Er<sup>3+</sup> was suppressed due to the strong absorption of IR1061. Nevertheless, H<sub>2</sub>O<sub>2</sub> may induce the destruction of IR1061, and thus weaken its absorption from 800 to 1,100 nm (Fig. 10a). As a result,  $I_{980}$  may gradually recover with increasing of the H<sub>2</sub>O<sub>2</sub> concentration. By contrast, the intensity of 1,180 nm emis-

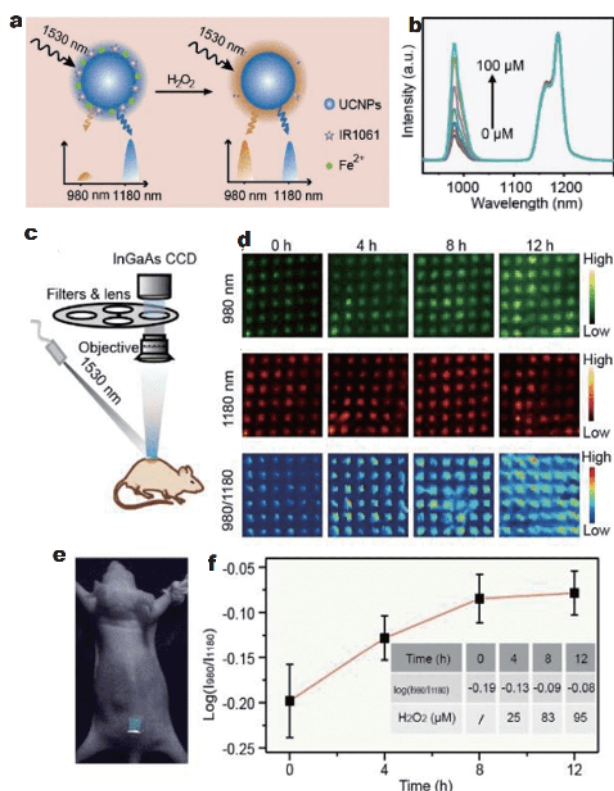
sion ( $I_{1180}$ ) of Ho<sup>3+</sup> was not affected by H<sub>2</sub>O<sub>2</sub>. Therefore, the concentration of H<sub>2</sub>O<sub>2</sub> can be quantified by determining the PL intensity ratio  $I_{980}/I_{1180}$  (Fig. 10b). As a proof-of-concept experiment, they fabricated the micro-needle patches based on the NaErF<sub>4</sub>:Ho@NaYF<sub>4</sub> NPs and IR1061 encapsulated polycaprolactone, which were applied for *in vivo* bioassay of H<sub>2</sub>O<sub>2</sub> in the inflammation site (Fig. 10c). By taking advantage of low autofluorescence of the NIR-II emission, the PL images of the microneedle array can be clearly observed under the skin tissue of mice. Upon excitation at 1,530 nm, the PL signal of 1,180 nm was stable while the signal of 980 nm gradually increased as the evolution of inflammation with the continuous generation of H<sub>2</sub>O<sub>2</sub> (Fig. 10d). According to the linear correlation between H<sub>2</sub>O<sub>2</sub> concentration and  $\log(I_{980}/I_{1180})$ , the concentration deviation of H<sub>2</sub>O<sub>2</sub> in the inflammatory site can be monitored from 0 to 12 h, which provides a feasible strategy for the quantitative detection of disease markers *in vivo*.

## TEMPERATURE SENSING

Luminescent nanothermometers are widely applied in nanomedicine, physiology, medical diagnosis, and controllable hyperthermia treatment. Particularly, luminescent nanothermometers, which are suitable for contactless, non-invasive temperature measurement at sub-cellular level, have gained much attention when it comes to photothermal therapeutics. Moreover, high-resolution temperature sensing is highly desired not only at the cellular level but also for *in vivo* disease diagnosis. As mentioned above, the NIR-II emission can penetrate much deeper in the biological tissue than visible or NIR-I emissions. Thus, nanothermometers with temperature-dependent emission in the NIR-II region are ideal candidates for temperature sensing at the deep tissue level [117].

The energy gap between some Stark sublevels of Ln<sup>3+</sup> ions is very small (only few tens of wavenumbers), which is strongly thermally coupled. Therefore, small temperature variations may result in remarkable changes in their emitting intensity due to the population redistribution of the thermally coupled energy levels. Such temperature dependent population of excited electrons in different energy levels of Ln<sup>3+</sup> allows for ratiometric nanothermometry based on the intensity ratio of two emission bands.

Among all the Ln<sup>3+</sup>-doped NIR-II nanothermometers, Nd<sup>3+</sup>-based NPs (e.g., YVO<sub>4</sub>:Nd [120], Gd<sub>2</sub>O<sub>3</sub>:Nd [121], YAlO<sub>3</sub>:Nd [122], LaF<sub>3</sub>:Nd@LaF<sub>3</sub>:Yb [118],) received the greatest attention. One of the most representative ex-

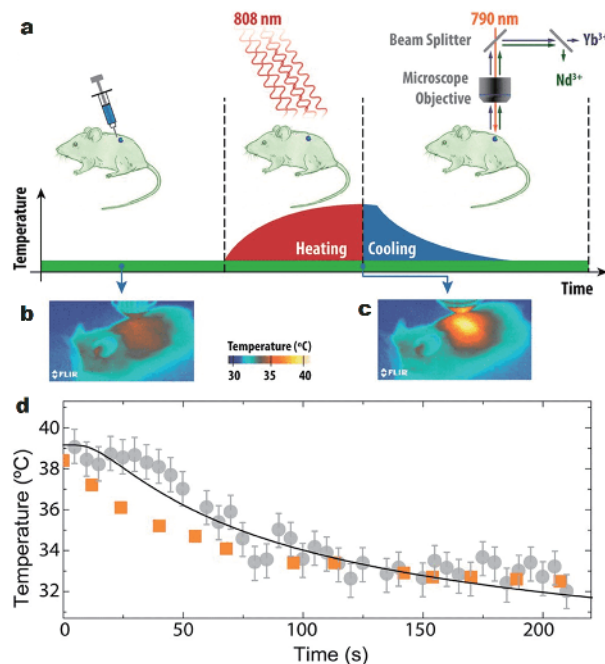


**Figure 10** (a) Schematic illustration of ratiometric luminescent assay for H<sub>2</sub>O<sub>2</sub>. (b) UC emission spectra of NaErF<sub>4</sub>:Ho@NaYF<sub>4</sub> NPs and IR1061 encapsulated polycaprolactone upon addition of different concentrations of H<sub>2</sub>O<sub>2</sub>. (c) Schematic illustration of the experiment for *in vivo* bioassay of H<sub>2</sub>O<sub>2</sub>. (d) UC luminescence images at 980 nm (top), 1,180 nm (middle), and the ratio of 980 to 1,180 nm (bottom) of microneedle patches taken at different times after lipopolysaccharide induced inflammation. (e) Photograph of the mouse treated with the microneedle patch. (f) Ratiometric luminescence of microneedle patches at different times with corresponding H<sub>2</sub>O<sub>2</sub> concentration. Reprinted with permission from Ref. [87]. Copyright 2018, Wiley-VCH Verlag GmbH & Co. KGaA.

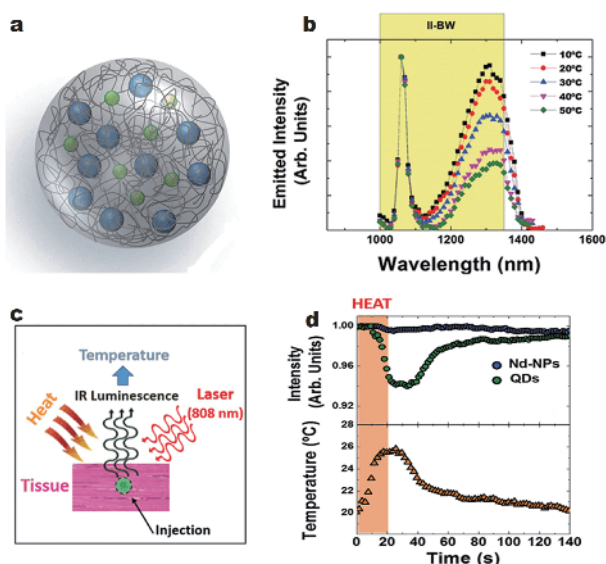
amples of Nd<sup>3+</sup>-based nanothermometers was reported by Kolesnikov and co-workers [120], who synthesized YVO<sub>4</sub>:Nd with NIR-II emission band from <sup>4</sup>F<sub>3/2</sub> → <sup>4</sup>I<sub>11/2</sub> of Nd<sup>3+</sup> for ratiometric temperature sensing. The integrated intensity ratio of 1,062 and 1,072 nm decreased from 25 to 55°C with thermal sensitivity of ~0.2%/°C. Carlos and co-workers proposed another kind of NIR-II nanothermometer based on Gd<sub>2</sub>O<sub>3</sub>:Nd NPs with temperature sensitivity of ~0.23%/°C, which was assessed by the ratio between the integrated intensity of the transitions originated from the highest- and lowest-energy Stark sublevels from <sup>4</sup>F<sub>3/2</sub> of Nd<sup>3+</sup> [121]. Although some progress of NIR II nanothermometry was gained in the past decade, the sensitivity of such nanothermometers was relatively low.

To this regard, Ximendes *et al.* [118] designed LaF<sub>3</sub>:Nd@LaF<sub>3</sub>:Yb core/shell NPs as nanothermometers upon the excitation at 790 nm. In their work, the integrated intensity ratio of Yb<sup>3+</sup> and Nd<sup>3+</sup> NIR-II emissions was found to decrease with the temperature from 10 to 50°C. A 4-fold higher sensitivity (0.44%/°C) was achieved than that of LaF<sub>3</sub>:Nd/Yb core-only NPs. Moreover, the proposed nanothermometer enabled monitoring of the real-time subcutaneous temperature (Fig. 11). After laser-induced heating, the subcutaneous temperature variation can be identified by the luminescent nanothermometry in a living animal (Fig. 11d), thus demonstrating the promise of Ln<sup>3+</sup>-doped NIR-II nanothermometer in sub-tissue temperature monitoring.

To further improve the sensitivity, other cations like Cr<sup>3+</sup> and Mn<sup>2+</sup> were also introduced into nanothermometry, due to their highly sensitive temperature dependent emission [106,123]. For example, nanothermometers based on the integrated intensity ratio of Cr<sup>3+</sup> and Nd<sup>3+</sup> emissions from LiLaP<sub>4</sub>O<sub>12</sub>:Cr/Nd NPs presented an outstanding sensitivity of 4.89%/°C, which was one order of magnitude higher than that of the vast majority of lu-



**Figure 11** (a) Schematic diagram of subcutaneous temperature sensing of mouse based on LaF<sub>3</sub>:Nd@LaF<sub>3</sub>:Yb core/shell NPs with 808-nm laser as heating source. Thermal images of mouse (b) before and (c) after heating treatment. (d) Time evolution of the subcutaneous temperature measured by luminescent nanothermometer (gray) and skin temperature measured by IR thermal camera (orange), respectively. Adapted with permission from Ref. [118]. Copyright 2016, American Chemical Society.



**Figure 12** (a) Schematic illustration of the nanostructure consisting of  $\text{NaGdF}_4\text{:Nd}^{3+}$  and  $\text{PbS/CdS/ZnS}$  QDs. (b) Temperature-dependent emission spectra of the hybrid nanocomposites upon excitation at 808 nm. (c) Schematic diagram of *in vitro* temperature measurement based on the hybrid nanocomposites upon excitation at 808 nm. (d) Evolution of the emission intensity of  $\text{NaGdF}_4\text{:Nd}$  NPs and  $\text{PbS/CdS/ZnS}$  QDs in the hybrid nanocomposites injected in chicken breast tissue (top) and temperature evolution of tissue (bottom) determined by the hybrid nanocomposites during a heating/cooling cycle. Reprinted with permission from ref. [119]. Copyright 2015, Wiley-VCH Verlag GmbH & Co. KGaA.

minescent thermometers. However, the emission of  $\text{Cr}^{3+}$  ions (820–840 nm) located in NIR-I region, which to some extent restricted their applications for temperature sensing *in vivo*.

Very recently, several interesting studies were reported by employing the hybrid structured nanothermometers for sensitive temperature sensing [119,124–127]. Rodriguez *et al.* [119] designed a hybrid nanothermometer for subtissue temperature sensing (Fig. 12). This hybrid nanothermometer was prepared following a double-emulsion encapsulation procedure by  $\text{PbS/CdS/ZnS}$  QDs as temperature-sensitive response unit and temperature-insensitive  $\text{NaGdF}_4\text{:Nd}$  as internal standard unit for the deep tissue ratiometric thermal sensing (Fig. 12b). The thermal sensitivity of the developed hybrid nanostructures was determined to be 2.5%/°C, which was one order of magnitude higher than that of the available NIR-I nanothermometers [128,129]. Subsequently, Xu *et al.* [124] further improved the thermal sensitivity. Triplet-triplet annihilation (TTA) dyad was modified on the surface of  $\text{NaYF}_4\text{:Nd}$  to design an organic/inorganic hybrid ratiometric thermometer (Fig. 13). The proposed

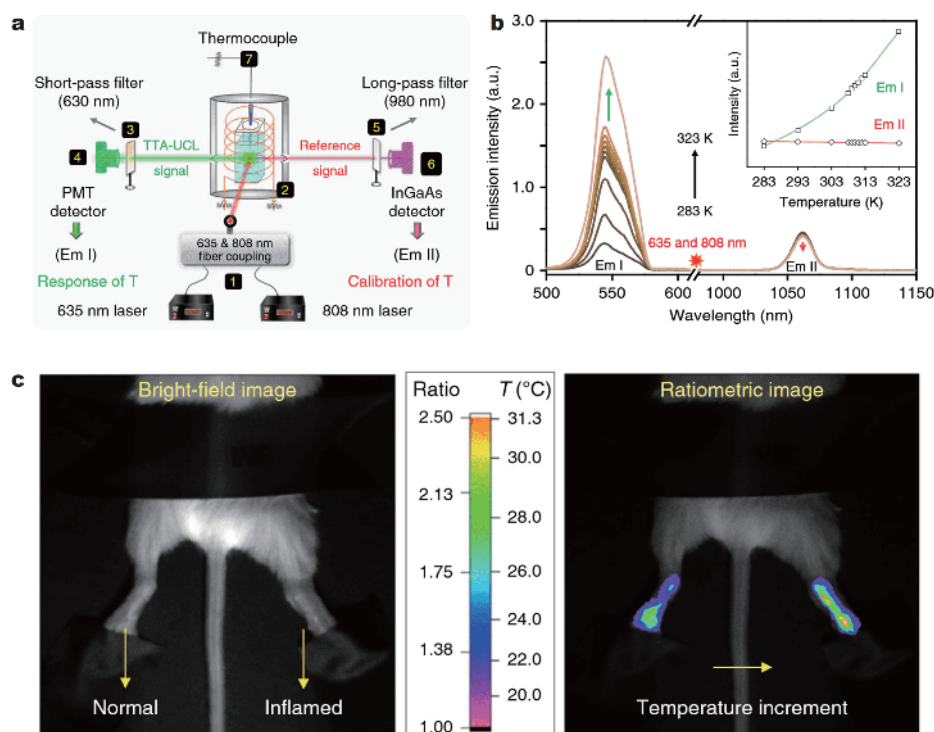
organic/inorganic hybrid nanothermometer was applied to monitor the specific temperature variations and map the temperature distributions in the inflammatory mode, which exhibited high thermal sensitivity ( $\sim 7.1\%/^\circ\text{C}$ ) and resolution ( $\sim 0.1^\circ\text{C}$ ). Nevertheless, the emission of TTA lay in the visible region, and double beam excitations combined with two detectors (PMT and InGaAs detectors) were required (Fig. 13a). In addition, different attenuation through tissue of the emission signal of 540 nm from TTA and 1,060 nm from  $\text{NaYF}_4\text{:Nd}$  might deteriorate the measurement accuracy *in vivo*.

## CONCLUSIONS AND PROSPECTS

$\text{Ln}^{3+}$ -doped NIR-II NPs, emerging as a novel class of luminescent probes and a promising alternative to conventional NIR-II probes, have gained substantial attention in recent years. Their optical performance can be modulated and PL efficiency can be improved *via* host selection, intentional cation incorporation, or surface modification with inorganic/organic layers. As a result, a series of highly efficient  $\text{Ln}^{3+}$ -doped NIR-II probes have been developed. By taking advantage of the distinct optical features of  $\text{Ln}^{3+}$  ions along with the large penetration depth and low autofluorescence of NIR-II emission in biological media, these  $\text{Ln}^{3+}$ -doped NIR-II NPs have been exploited for various applications in bioimaging, bioassay and temperature sensing.

Despite these encouraging achievements, it remains challenging for the practical bioapplications of these  $\text{Ln}^{3+}$ -doped NIR-II nanoprobe. One of the key bottlenecks that prevent the utilization of  $\text{Ln}^{3+}$ -doped NIR-II nanoprobe is their poor QYs caused by low absorption cross-section of  $\text{Ln}^{3+}$ , since low PL QY may greatly reduce the SNR of bioimaging and the sensitivity of bioassay. Most of the host materials are relatively inert in the luminescence process of  $\text{Ln}^{3+}$  emitters, leaving large room for improvement. Therefore, future efforts should be made to explore more innovative host materials and sensitization strategies aiming to improve the NIR-II QY of  $\text{Ln}^{3+}$ -doped NPs. Additionally, the ultimate goal in this field is to develop commercial assay kits based on  $\text{Ln}^{3+}$ -doped NIR-II nanoprobe for *in vivo* disease theranostics. Although several kinds of  $\text{Ln}^{3+}$ -doped NPs have been applied as nanoprobe for *in vivo* bioimaging, currently it still remains unexplored for precise bioassay *in vivo*. It is highly desired to design and develop novel heterogeneous/homogeneous assay techniques based on  $\text{Ln}^{3+}$ -doped NIR-II nanoprobe for rapid, sensitive and specific bioassay of target analytes *in vivo*. Moreover, the narrow emission bands and tunable PL lifetimes of  $\text{Ln}^{3+}$ -doped





**Figure 13** (a) Schematic representation of experimental device for temperature sensing based on TTA-modified  $\text{NaYF}_4:\text{Nd}$  NPs via double beam excitations (635 and 808 nm) and two detectors (PMT and InGaAs detectors). (b) Temperature-dependent emission spectra of TTA-modified  $\text{NaYF}_4:\text{Nd}$  NPs from 283 to 323 K. Inset shows the temperature dependence of the integrated emission intensities of TTA and NIR-II emission from  $\text{Nd}^{3+}$ . (c) Bright field image (left) and the ratiometric image based on TTA-modified  $\text{NaYF}_4:\text{Nd}$  NPs in an inflammatory mode (right) showing temperature distributions in the two legs of a mouse. Adapted with permission from Ref. [124]. Copyright 2018, Nature Publishing Group.

NPs provide unique opportunities for simultaneous multiplexed assay of different disease markers in deep tissue. Last but not the least, the prevailing *in vivo* imaging systems are equipped with a silicon-based charge-coupled device or complementary metal oxide semiconductor camera, which is unusable for NIR-II imaging studies. To demonstrate the potential bioapplications of  $\text{Ln}^{3+}$ -doped NIR-II NPs, several research groups proposed different custom-built systems. As such, it is difficult to compare and evaluate the results from worldwide laboratories. There is a growing demand for the development of new-generation, but commercial cost-effective systems with highly NIR-sensitive detectors, which may significantly promote the clinical applications of NIR-II nanoprobes for sensitive bioimaging and bioassays in disease diagnosis.

Received 31 January 2019; accepted 13 March 2019;  
published online 17 April 2019

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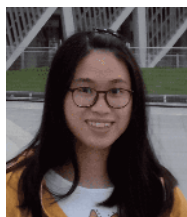
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**Acknowledgements** This work was supported by the Strategic Priority Research Program of the CAS (XDB20000000), the National Natural Science Foundation of China (21771185, 11704380, 51672272, 21804134 and U1805252), the CAS/SAFEA International Partnership Program for Creative Research Teams, and the Natural Science Foundation of Fujian Province (2017I0018).

**Author contributions** Yu S, Tu D and Chen X conceived and wrote the manuscript and designed the figures. Lian W and Xu J prepared the materials of bioapplications. All authors contributed to the general discussion and revision of the manuscript.

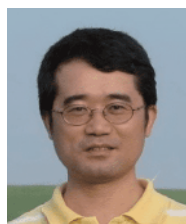
**Conflict of interest** The authors declare no conflict of interest.



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## 稀土掺杂近红外二区发光纳米探针及其生物应用

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**摘要** 近红外二区(1000–1700 nm)荧光纳米探针可以显著降低穿透组织时的光散射和自荧光效应的影响, 从而提高探测深度以及成像分辨率. 目前已报道的近红外二区生物探针主要基于有机荧光团、碳纳米管、量子点以及共轭聚合物. 稀土离子掺杂纳米晶因其优异的发光性质, 被认为是一类极具发展潜力的生物探针. 本文从设计高效近红外二区发光的稀土掺杂纳米材料的角度出发, 主要介绍了此类稀土纳米探针的基质选择、阳离子掺杂和表面修饰等设计策略的研究进展, 及其在无背景生物成像、高灵敏生物检测和温度探测等领域的最新应用. 此外, 还展望了此类荧光纳米探针面临的挑战以及未来发展趋势.