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Er\textsuperscript{3+} Sensitized 1530 nm to 1180 nm Second Near-Infrared Window Upconversion Nanocrystals for \textit{in Vivo} Biosensing

Lu Liu, Shangfeng Wang, Baozhou Zhao, Peng Pei, Yong Fan, Xiaomin Li*, Fan Zhang*

Abstract: Fluorescent bioimaging in the second near-infrared window (NIR-II) can probe deep tissue with minimum auto-fluorescence and tissue scattering. However, all the current NIR-II fluorophores related in vivo biodetection are only focused on direct disease lesion or organ bioimaging, it is still a big challenge to realize the NIR-II real-time dynamic biosensing. Herein, we present a new type of Er\textsuperscript{3+} sensitized upconversion nanoparticles with both excitation (1530 nm) and emission (1180 nm) located in NIR-II window for \textit{in vivo} biosensing. Significantly, the microneedle patch sensor for \textit{in vivo} inflammation dynamic detection is developed based on the ratiometric fluorescence by combining the effective NIR-II upconversion emission and H\textsubscript{2}O\textsubscript{2} sensing organic probes under the Fenton catalysis of Fe\textsuperscript{2+}. Due to the large anti-Stokes shifting, low auto-fluorescence and tissue scattering of the NIR-II upconversion luminescence, the inflammation can be dynamically evaluated \textit{in vivo} at very high resolution (200 * 200 μm).

Currently, optical fluorescence imaging is highly attractive for \textit{in vivo} observing and assessing biological processes because of its high spatial and temporal resolution.\textsuperscript{[1]} For \textit{in vivo} biological imaging through optically turbid tissues, photon penetration depth is mainly determined by scattering and absorption of tissue components, while noise and background levels result from tissues auto-fluorescence. Compared to the fluorescence imaging at visible (400 ~ 700 nm) and first near-infrared (NIR) window region (NIR-I; 700 ~ 900 nm), bioimaging in the second NIR window (NIR-II, 1000~1700 nm) has been demonstrated successfully with better spatial resolution and feature contrast at deeper imaging depth due to reduced auto-fluorescence and photon scattering.\textsuperscript{[2]} The NIR-II imaging technique has been established and improved for \textit{in vivo} imaging using a series of materials with fluorescence emission in the NIR-II window, such as single-walled carbon nanotubes, quantum dots, rare-earth doped nanoparticles and organic dyes, etc.\textsuperscript{[3]}

Lanthanide based upconversion nanoparticles (UCNPs), which can convert lower energy photons into high energy emissions, provide the potential for NIR remote \textit{in vivo} biosensing.\textsuperscript{[4]} Especially, the biosensor by integrating UCNPs and organic sensing chromophores endows the outstanding recognition properties of chromophores and remarkable optical properties of UCNPs, thus creating highly sensitive sensors to detect various analytes.\textsuperscript{[5]} However, one of the limitations for the commonly used Yb\textsuperscript{3+} and Nd\textsuperscript{3+}-sensitized UCNPs is the fixed excitation bands in the NIR-I (980 nm for Yb\textsuperscript{3+} and 800 nm for Nd\textsuperscript{3+}),\textsuperscript{[6]} which impede further improvement of the fluorescent imaging and sensing performance. Recently, Chan \textit{et al.} successfully developed an energy-looping UCNPs with 1064 nm NIR-II excitation, however, the emission is still located in NIR-I region (800 nm).\textsuperscript{[7]} On the other hand, since the broad absorption spectra of most of the NIR sensing chromophores (~600 - 1100 nm), it's difficult to realize the NIR-II sensor by using regular NIR-II materials. Because the excitation wavelengths for almost all of reported NIR-II materials are located around 600 - 1100 nm, which are overlapping with the absorption of the NIR chromophores.

Herein, we present a new type of Er\textsuperscript{3+} sensitized upconversion nanoparticles with both excitation and emission located in NIR-II window for the first time. In the simple core/shell structured NaErF\textsubscript{4}:Ho@NaYF\textsubscript{4} nanoparticle, the Er\textsuperscript{3+} are acting as both sensitizer and emitter to harvest pump photons at 1530 nm and subsequently promote the 980 nm emission, while the Ho\textsuperscript{3+} dopants can also serve as emitter to generate an efficient upconversion emission at 1180 nm. Significantly, the microneedle patch sensor for \textit{in vivo} inflammation dynamic detection is developed based on the ratiometric fluorescence (I\textsubscript{980}/I\textsubscript{1180}) by combining the effective NIR-II upconversion emission and H\textsubscript{2}O\textsubscript{2} sensing organic chromophore probe IR1061 under the Fenton catalysis of Fe\textsuperscript{2+}. Owing to the large anti-Stokes shifting, low auto-fluorescence and tissue scattering of the NIR-II upconversion luminescence, the inflammation can be imaged and evaluated \textit{in vivo} at very high resolution (200 * 200 μm).

Er\textsuperscript{3+} is known for its upconversion optical activity in the visible region, and \textsuperscript{1}I\textsubscript{15/2} → \textsuperscript{1}F\textsubscript{9/2}, \textsuperscript{1}I\textsubscript{11/2} transition offers a strong downshifting luminescence.\textsuperscript{[8]} Notably, it has multiple excitation bands from visible to NIR-I and NIR-II region, such as 650, 980, and 1530 nm (Figure S1), corresponding to transitions from \textsuperscript{1}I\textsubscript{11/2} to \textsuperscript{4}F\textsubscript{9/2}, \textsuperscript{1}I\textsubscript{15/2}, \textsuperscript{4}F\textsubscript{9/2}, \textsuperscript{4}F\textsubscript{7/2}, \textsuperscript{4}F\textsubscript{5/2}, \textsuperscript{1}I\textsubscript{9/2}, \textsuperscript{1}I\textsubscript{7/2}.
and $^4I_{13/2}$ (Figure 1A), respectively. It can be seen that the absorption intensity of Er$^{3+}$ at 1530 nm is about two times higher than that of at 980 nm. It is found that the Er$^{3+}$ possesses equal energy gap of 6500 cm$^{-1}$ ($^4I_{15/2}$$^4I_{13/2}$$^4I_{11/2}$$^4I_{9/2}$) between each excited energy level (Figure 1A), which exactly correspond to the strong absorption of Er$^{3+}$ at 1530 nm. This typically ladder-like arranged energy levels are the essential requirement for the upconversion process.[9] In addition, it has been reported that the quenching effect at high Er$^{3+}$ doping concentration is the surface defects, rather than cross-relaxation between Er$^{3+}$ dopants.[8] It means that the Er$^{3+}$ ions can be doped at very high concentration with low cross-relaxation, further support the feasibility of the fabrication of Er$^{3+}$ sensitized NIR-II UCNPs.

As a proof of concept, the NaErF$_4$ is used as matrix to sensitize the Ho$^{3+}$ activator (NaErF$_4$:Ho), followed by coating with an inert NaYF$_4$ shell to suppress the surface quenching (Figure 1A). Transmission electron microscopy (TEM) image shows discernible contrast for the core/shell structured UCNPs with an apparent uniform size (24.7 nm) and morphology (Figure 1B, S2). The high-angle annular dark field scanning TEM (HAADF-STEM) was also employed to identify core-shell structures, in which the brighter regions correspond to the heavier Er element and the darker parts correspond to the lighter one (Y). The high-resolution TEM (HRTEM) image shows that the nanoparticles are highly crystalline hexagonal phase, which is consistent with the result of X-ray diffraction (XRD) patterns (Figure S3).

Under the excitation of 1530 nm, the obtained NaErF$_4$:2%Ho@NaYF$_4$ UCNPs show three strong upconversion emission peaks at 650 (F$_{9/2}$$^4I_{15/2}$), 980 (F$_{11/2}$$^4I_{15/2}$) and 1180 nm (F$_{8}$$^4I_{13/2}$) (Figure 1C). There are multiple energy transfer mechanisms working together to realize this unique Er$^{3+}$ sensitized upconversion emissions: the excited state absorption (ESA) upconversion process for the Er$^{3+}$ dominant emissions at 650 and 980 nm; the energy transfer upconversion (ETU) process for the Ho$^{3+}$ dominant emission at 1180 nm. The intensity of the upconversion luminescence is significantly enhanced with increasing of NaYF$_4$ inert shell thickness, and nearly saturated when the thickness increased to 4 nm (Figure S5). According to the power law I = AP$^n$, (I is the upconversion intensity; A is constant; P is the excitation power), the number of pumping photons ($n$) required to excite the emission centers from ground state to emitting state can be easily calculated. The slope values of 2.11, 1.31 and 1.36 were observed for the 650, 980, and 1180 nm emissions (Figure S6), respectivley, illustrating that three photon process were involved to generate 650 nm emission and two photon process for 980 and 1180 nm emission (Figure 1A).

To further verify the highly efficient ETU process for the NIR-II upconversion luminescence, the energy transfer between Er$^{3+}$ sensitizer and Ho$^{3+}$ activator is blocked by designing of the core/shell structure (Figure 2A). Compared with the NaErF$_4$:Ho@NaYF$_4$ nanoparticles, the upconversion emissions of Ho$^{3+}$ are sharply reduced by more than 60 times for the NaErF$_4$:Ho@NaYF$_4$ and NaErF$_4$:2%Yb,2%Ho@NaYF$_4$ nanocrystals under 980 and 1530-nm excitations. The close relationship between the concentration of Er$^{3+}$ and Ho$^{3+}$ activators monotonically increased with increasing of NaYF$_4$ inert shell thickness, and nearly saturated at the concentration of Er$^{3+}$ (Figure 2D, S10). So, we considered that the concentration quenching effect of Er$^{3+}$ would not dominate the quenching process, even in the heavily Er$^{3+}$ doping concentration, which is consistent with the literature report.[8] In comparison, concentration quenching effect of Ho$^{3+}$ activator for the upconversion emission is much stronger: when the Ho$^{3+}$

As shown in Figure 2C, the upconversion emission dominated by Ho$^{3+}$ activators monotonically increased with increasing of Er$^{3+}$ doping concentration, indicating higher Er$^{3+}$ concentration can harvest more pump photons to excited states and finally transfer to the Ho$^{3+}$ ions. Under the excitation of 1530 nm, all of the three upconversion emissions at 650, 980, and 1180 nm exhibit long luminescence lifetimes with surprisingly little dependence on the doping concentration of Er$^{3+}$ (Figure 2D, S10). So, we considered that the concentration quenching effect of Er$^{3+}$ would not dominate the quenching process, even in the heavily Er$^{3+}$ doping concentration, which is consistent with the literature report.[8] In comparison, concentration quenching effect of Ho$^{3+}$ activator for the upconversion emission is much stronger: when the Ho$^{3+}$

![Figure 2](image-url)

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doping concentration is higher than 2%, the upconversion emissions at 650, 980, 1180 nm dominated by both Er3+ and Ho3+ are greatly quenched (Figure 2E). Correspondingly, all the life time of the upconversion emissions at 650, 980, 1180 nm sharply decreased (Figure 2F). In contrast, when the Ho3+ doping concentration is lower than 2%, the intensity (Figure 2E) and lifetime (Figure S10) of the upconversion emission at 980 nm and 650 nm dominated by Er3+ is gradually decreased as increasing of Ho3+ doping concentration. Meanwhile, the upconversion emission at 1180 nm dominated by Ho3+ is greatly increased, demonstrating the energy transfer between Er3+ (4I11/2) and Ho3+ (5I6). The Er3+ sensitized upconversion process can also be applied to Nd3+ activators. To avoid the quenching effect induced by the energy back-transfer from Nd3+ (4I15/2) to Er3+ (4I11/2) (Figure 2G),[6d] a core/shell structured NaErF4@NaYF4:Nd@NaYF4 that separates the Nd3+ activators and Er3+ sensitizers was fabricated. The NIR-II upconversion luminescence at 1060 nm dominated by Nd3+ can be obtained under excitation of 1530 nm (Figure 2H), which is much stronger than that of the Nd3+ and Er3+ co-doping core/shell structure (NaErF4:Nd@NaYF4) (Figure S12A).

Taking the advantage of the multiple emissions and large anti-Stokes shifting (> 350 nm), the obtained NIR-II UCNPs can be used for the ratiometric fluorescent sensor. In comparison with the emission at 650 nm, emissions at 980 and 1180 nm exhibit similar attenuation kinetics in the simulation tissue, which is very important for the in vivo ratiometric fluorescent sensor (Figure 3A, B). Combined with the selective absorption property at 980 nm and the H2O2 sensitivity of IR1061 in Fenton reaction (Figure S13, S14), the ratiometric fluorescent (I980/I1180) of the probe in response to varying H2O2 concentration.

Owing to the well processability of PCL matrix, the sensor can be easily processed into various shapes. As a model experiment, microneedle patches were fabricated for imaging of skin inflammation by casting PCL, NIR-II UCNPs, IR1061 and Fe2+ complex solution into inverted polydimethylsiloxane (PDMS) mold to form the needle tips (Figure 4A).[10] A top layer of pure PCL solution was drop-wise added onto the mold to create a stable substrate (Figure S18). The obtained microneedles are arranged in a 10 × 10 array with an area of 8 × 8 mm. Each needle is
pyramid-shaped with a base radius of 200 μm and a height of 600 μm (Figure 4B). The confocal laser scanning microscopy of microneedle patch showed that the NIR-II UCNP s were homogeneously loaded in the microneedles (Figure 4C, D). Then the microneedle patches were imaged at different concentration of H2O2 under the excitation of 1530 nm. Consistent with the result of the upconversion luminescence spectra (Figure 3D), the luminescent imaging statistical results of the microneedle arrays did not show obvious change in 1180-nm channel (Figure 4G, H). In comparison, the brightness of the needles in 980-nm channel dramatically increased with increasing of H2O2 concentration (Figure 4E, F). The linear correlation between the Log (I980/I1180) and H2O2 concentration is well maintained (Figure 4I, J).

In summary, Er3+ sensitized NaErF4:Ln3+@NaYF4 UCNPs (Ln3+ = Ho3+ or Nd3+) were synthesized for the first time. The NIR-II upconversion emissions with large anti-Stokes shifting of Er3+ (980 nm), Ho3+ (1180 nm) and Nd3+ (1060 nm) can be achieved by the well designed core/shell structure, optimized the doping concentration and the shell layer thickness under the 1530 nm irradiation. Based on the ratiometric fluorescent (I980/I1180) of the Er3+ sensitized UCNPs, the microneedle patch is prepared and subjected for in vivo dynamic inflammation sensing at very high resolution (200 * 200 μm). We considered that this study may open up new avenues for the development of NIR-II nanoprobes for high resolution biosensing.

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Keywords: NIR-II imaging • upconversion nanoparticles • microneedle • biosensing


Er$^{3+}$ sensitized NIR-II upconversion nanoparticles with both excitation and emission in NIR-II region has been synthesized for \textit{in vivo} imaging for the first time.

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