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Ratiometric Afterglow Nanothermometer for Simultaneous in Situ Bioimaging and Local Tissue Temperature Sensing

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ABSTRACT: Simultaneous in situ bioimage tracing and temperature sensing have been two of the foci of modern biomedicine that have given birth to designing novel luminescent nanothermometers with dual functions. To minimize the disadvantages of existing approaches, like the surface effect of nanoparticles, autofluorescence, and/or the thermal effect described herein, a bifunctional (simultaneous in vivo bioimaging and local tissue temperature sensing) ratiometric afterglow nanothermometer has been realized in the physiological temperature range (298–325 K) based on persistent luminescent Cr3+ (with d electron configuration)-doped zinc gallogermanate nanoparticles. The contributions of the radiative 2E → 2A2 and 4T2 → 4A3 transitions of surface and interior Cr3+ to the near-infrared afterglow dual emissions are modeled, and the measured thermal sensitivities (0.043–0.047 K−1) for detecting the temperature of a human serum albumin solution are 1 order of magnitude higher than those using an upconverting luminescent nanoparticle strategy. For a local tissue with various thicknesses (0–15 mm), a similar detection sensitivity can be obtained because of the use of the near-infrared wavelength. Meanwhile, in situ temperature sensing can recur after near-infrared light (808 nm) re-excitation.

1. INTRODUCTION

Recently, luminescent nanothermometers (LNTHs) have been attracting a great deal of research interest because of their potential applications in biomedicine and particularly intracellular thermal sensing because of the simplicity of their use in detection, high signal-to-noise ratio, real-time reading, etc.1–9 On the basis of the temperature-dependent luminescence under ultraviolet (UV), visible, and near-infrared (NIR) light excitation, band shape, spectral position, polarization, lifetime, and/or intensity ratio, the LNTHs can be used for in situ temperature measurement.5,6,9 For example, Kalyuchuk et al.10 designed a carbon dot nanothermometer based on the temperature-dependent photoluminescence lifetimes (λem = 421 nm) under UV excitation (λex = 385 nm). Under visible light excitation (λex = 405 nm), Chen et al.11 developed a novel water-soluble dual-emissive (λem/λexc) phosphorescent polymeric thermometer. On the basis of the temperature-dependent photoluminescence intensity (Iλem/λexc) or lifetime, Zhegalova et al.12 designed a nanocapsule ratiometric luminescent nanothermometer under visible and NIR light excitation (λex = 560 and 740 nm, respectively). Furthermore, Ruiz et al.13 successfully operated nanothermometers in the second biological window (1000–1350 nm) using Ag/Ag2S nanocrystals as multiparametric thermal sensing probes under NIR light excitation (λex = 822 nm). In particular, the ratiometric luminescent nanothermometers (RLNTHs) using upconversion nanoparticles (UCNPs) have exhibited some unique features, such as high photochemical stability, high sensitivity, and reliability among all kinds of LNTHs.14–16 At present, NIR UCNPs have been successfully used for intracellular and local tissue thermal detection with high accuracy and high penetration because both excitation and emission wavelengths fall in the so-called “first biological window” (650–1000 nm).14,17–19 The concerns of the aforementioned strategies are to reduce the possible autofluorescence under external excitation and local thermal effect induced by NIR excitation light,20–23 to realize the temperature sensing of moving targets, e.g., blood circulation, and to further improve the thermal sensitivity of nanothermometers.

On the other hand, novel Cr3+-doped zinc gallate and gallogermanate NIR superlong (at least several hours) afterglow nanoparticles, such as ZnGa2O4:Cr3+, Zn1.1Ga1.8Ge0.1O4:Cr3+, Eu3+@SiO2, and Zn2.9Ga1.96Ge2O10:Cr3+/Pr3+, have recently been employed for in vivo imaging in an animal model under non-excitation conditions.24–28 Considering that the Cr3+ ion with a d electron configuration (which is sensitive to its external surroundings) has two thermally coupled levels, namely, 2E

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and $^{4}T_{2}(F)^{29}$, the afterglow dual-emission intensity (over several hours) ratio might be acquired. In addition, for the RLNTHs using UCNPs, the contributions of radiative transitions from two thermally coupled levels of both surface and interior rare earth ions to the luminescence cannot be distinguished because of the luminescent centers with an $f$ electron configuration, which is insensitive to their external surroundings. Thus, it is wise to avoid the use of rare earth ions and is essential to comprehend how the luminescent centers located on the surface of nanoparticles influence the sensitivity and reliability of LNTHs. Furthermore, when NIR afterglow nanoparticles are applied to temperature sensing and image tracing, X-ray intermittent excitation can even significantly enhance the afterglow emission and increase the quality of bioimaging.30 Despite these great advantages of NIR superlong afterglow nanoparticles for local tissue temperature sensing, no relevant report has yet appeared.

In this paper, we report for the first time the design of a bifunctional (simultaneous in vivo bioimaging and local tissue temperature sensing) NIR ratiometric afterglow nanothermometer (RANTH) based on Cr$^{3+}$-doped zinc gallogermanate (ZGGO:Cr$^{3+}$). A theoretical model related to temperature sensing was proposed, taking into account the contributions of the radiative $^5E\rightarrow^4A_2$ and $^3T_2\rightarrow^4A_2$ transitions of the surface and interior Cr$^{3+}$ to the NIR afterglow emission. For the application of ZGGO:Cr$^{3+}$ NIR RANTHs in the detection of the temperature of a human serum albumin (HSA) solution, the measured thermal sensitivity ($0.043-0.047$ K$^{-1}$) is 1 order of magnitude higher than that using the upconverting luminescent nanoparticle strategy. A similar detection sensitivity can be maintained for thermal sensing of local tissue as deep as 15 mm. Meanwhile, in situ temperature sensing can recur after NIR light re-excitation.

2. EXPERIMENTAL SECTION

2.1. Synthesis of the ZGGO:Cr$^{3+}$ NIR RANTH. The ZGGO:Cr$^{3+}$ NIR RANTH was prepared using a normally hydrothermal path and following a subsequent vacuum annealing method as described in our previous report.$^{37}$ The raw materials, Zn(CH$_3$COO)$_2$, Ga(NO$_3$)$_3$, xH$_2$O, GeCl$_4$, and Cr(NO$_3$)$_3$$\cdot$9H$_2$O, were well blended in deionized water according to a 2:2.98:0.75:8:0.02 Zn:Ga:Ge:Cr mole ratio. After the mixture had been heated for 4 h at 453 K in a steel autoclave, the precipitates appeared as the temperature was decreased. Subsequently, the precipitates were dried at 353 K for 4 h after being washed with deionized water. Under vacuum, the final ZGGO:Cr$^{3+}$ nanoparticles were acquired by annealing the precipitates at 1073 K for 1.5 h.

2.2. Characterization. The crystal structure of the ZGGO:Cr$^{3+}$ RANTH was examined by an X-ray diffraction (XRD) spectrometer.

Figure 1. (a) X-ray diffraction pattern and (b) transmission electron microscopy image of ZGGO:Cr$^{3+}$ nanoparticles. The inset in panel b shows a selected area electron diffraction pattern. (c) Normalized excitation ($\lambda_{em}=696$ nm) and emission ($\lambda_{ex}=256$ nm) spectra of ZGGO:Cr$^{3+}$ nanoparticles. (d) Afterglow decay curves of ZGGO:Cr$^{3+}$ nanoparticles monitored at 696 nm after stopping 5 min X-ray and UV light (256 nm) irradiations. The inset in panel d shows the afterglow spectra acquired with a decay time of 900 min. (e) Images of the ZGGO:Cr$^{3+}$ nanoparticles under white light taken at different decay times after 5 min UV-lamp (254 nm) and X-ray irradiations.
Article

Figure 2. Schematic illustrations of the contributions of the interior and surface Cr$^{3+}$ ions to the temperature-dependent NIR luminescence of the ZGGO:Cr$^{3+}$ RANTH.

(Rigaku, D/max-RA) using Cu Kα radiation (line of 0.15418 nm). The single-crystal feature of RANTH is confirmed by a field emission transmission electron microscope (JEOL-2100F). The luminescence spectra were acquired with a spectrophotometer (Shimadzu, RF5301PC). The luminescence—kinetics measurements were performed by using a 600 MHz digital oscilloscope (LeCroy) and an optical parametric oscillator (OPO) system. Afterglow emission decay curves were recorded using a Zolix spectrophotometer (Omin-300) after X-ray and/or UV irradiation. ZGGO:Cr$^{3+}$ nanoparticles were pressed into pellets to measure thermally stimulated luminescence spectra monitored at 696 nm in the temperature range of 88–673 K. The heating rate was kept constant at 5 K/min. X-ray (molybdenum target) irradiation was performed on an X-ray machine (PHYWE). The irradiation dose rate is ∼11 Sv/h. The photostimulatable luminescence spectra were recorded on a RF5301PC instrument with an 808 nm laser as a source of stimulation.

2.3. In Vivo NIR Afterglow Imaging. The in vivo bioimaging experiments were performed using a mouse (C57BL/6, obtained from Liaoning Changsheng biotechnology Co., Ltd.) anesthetized with chloral hydrate (200 μL, 4%) in advance. ZGGO:Cr$^{3+}$ nanoparticles (10 mg/mL) dispersed in a 10 mM phosphate-buffered saline (PBS) solution were administered to the mouse through a subcutaneous injection. The solution containing ZGGO:Cr$^{3+}$ nanoparticles was irradiated for 5 min using a 254 nm UV lamp before injection. The in vivo NIR afterglow bioimages were recorded using a Berthold NightOWL LB 983 imaging system without an excitation source (emission filter, 700 nm; exposure time, 15 or 120 s).

2.4. Synthesis of ZGGO:Cr$^{3+}$@SiO$_2$ Nanocomposites. ZGGO:Cr$^{3+}$@SiO$_2$ nanocomposites were fabricated by a Stöber method. ZGGO:Cr$^{3+}$ nanoparticles (30 mg) were dispersed in ethanol (80 mL). After the solution was stirred for 1 h, 20 mL of deionized water and 0.1 g of tetraethyl orthosilicate were added dropwise. The pH of the solution was adjusted to 12 by adding an amount of the ammonia solution (28 wt %). After the mixed solution was stirred for 6 h and the sediments were centrifuged and washed with water, the final products (ZGGO:Cr$^{3+}$@SiO$_2$) were obtained.

2.5. In Vitro Cytotoxicity Assays. L929 mouse fibroblast cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal bovine serum. The cultured cells were plated in 96-well plates at a density of 4200 cells/well for 24 h at 37 °C in a humidified atmosphere (containing 5% CO$_2$). The attached cells were exposed to different concentrations (0, 12.5, 25, 50, and 100 μg/mL) of ZGGO:Cr$^{3+}$ and ZGGO:Cr$^{3+}$@SiO$_2$ for 24 h. Then, 10 μL of an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL) was added to each well, and plates were incubated at 37 °C in a humidified atmosphere (containing 5% CO$_2$) for 4 h. Finally, 100 μL of DMSO (dimethyl sulfoxide) was added to each well. The values of optical density (OD) at 490 nm were measured by a microplate reader, and the cell viability was calculated using the following equation: cell viability (%) = (absorbance value of treatment group/mean absorbance value of control) ∗ 100. All tests were performed in three independent experiments.

3. RESULTS AND DISCUSSION

3.1. Crystal Feature and NIR Afterglow Imaging of the ZGGO:Cr$^{3+}$ RANTH. To ascertain the crystallographic structure of the ZGGO:Cr$^{3+}$ RANTH, XRD characterization was performed as shown in Figure 1a. Compared with that of ZnGa$_2$O$_4$ (JCPDS File 38-1240), a similar pattern is presented, and no impurity phase appears. Because the radii of Cr$^{3+}$ and Ge$^{4+}$ ions (0.61 and 0.54 Å, respectively) are very close to that of the Ga$^{3+}$ ion (0.62 Å), they will substitute for the Ga$^{3+}$ ion and occupy the octahedral sites. To confirm its single-crystal feature, the typical transmission electron microscopy (TEM) micrograph and selected area electron diffraction of ZGGO:Cr$^{3+}$ nanoparticles are given in Figure 1b and the inset. The average particle size is ∼50 ± 11 nm, and a periodically aligned array pattern with sharp spots can be found as expected. The related histogram of a particle size distribution is shown in Figure S1.

To examine their optical properties, excitation and emission spectra of ZGGO:Cr$^{3+}$ nanoparticles were acquired as shown in Figure 1c. By monitoring the nanoparticles at 696 nm, we observed four broad absorption bands in the wavelength range of 220–660 nm, which could be attributed to the charge transfer band (CTB) of the Ga–O group and the $^4$A$_2$ → $^4$T$_1$($^4$P), $^4$T$_1$($^4$F), and $^4$T$_2$($^4$F) transitions of Cr$^{3+}$ ions. Upon 256 nm excitation, the emission spectrum shows a narrow band NIR emission at 696 nm superimposed on a broad NIR emission peak around 712 nm. According to the work of Grinberg et al. and Back et al., in the case of gallogermanate with an intermediate crystal field, the narrow and broad bands that can be assigned to the $^3$E → $^4$A$_2$ and $^4$T$_1$($^4$F) → $^4$A$_2$ transitions of Cr$^{3+}$ ions, respectively, appear simultaneously. Because a thermal equilibrium exists between the $^3$E and $^4$T$_1$ states, the relationship between the temperature and ratiometric luminescence can be established (vide infra).
After UV light (256 nm) or X-ray irradiation for 5 min, ZGGO:Cr³⁺ nanoparticles exhibited a long NIR afterglow time of >900 min (15 h) as shown in Figure 1d, which is intensive compared with UV excitation (inset of Figure 1d). To further demonstrate the capacity of in situ imaging without external excitation, the NIR afterglow images of the pellets (pressed by ZGGO:Cr³⁺ nanoparticles) were taken at different decay times after stopping 5 min UV light and X-ray beam irradiation (Figure 1e). Distinct NIR images could be obtained for more than 30 min. Furthermore, X-ray irradiation led to images that were brighter than those from UV excitation. Thus, X-ray excitation is more efficient for realizing high-quality NIR afterglow imaging.

3.2. Temperature-Dependent Dual Emissions and Luminescent Mechanism. The schematic presented in Figure 2 gives an overview of the temperature-dependent dual emissions. For a single nanoparticle under X-ray or UV excitation, NIR luminescence derives from the contributions of the ²E→⁴A₂ and ⁴T₁→⁴A₂ transitions of surface and interior Cr³⁺ ions. However, the emission spectra of ZGGO:Cr³⁺ nanoparticles at different temperatures can be fitted by three Gaussian peaks as shown in Figure 2, which is different from the expected number of four. According to the Tanabe–Sugano diagram for the Cr³⁺ ion (Figure S2), the ²E level of the Cr³⁺ ion is almost invariable with local crystal field strength, while the ⁴T₁(⁴F) level decreases with a decrease in the crystal field strength. Thus, the sharp emission (peak 1) at 695 nm can be ascribed to the total contributions of the ²E→⁴A₂ transitions of the surface and interior Cr³⁺ ions. Because the surface Cr³⁺ ion is located in a site with a crystal field that is weaker than that of the interior Cr³⁺ ion, the other two broad peaks originate from the ⁴T₂(⁴F)→⁴A₂(⁴F) transitions of the interior (peak 2) and surface (peak 3) Cr³⁺ ions.

To comprehend the kinetic process of the temperature-dependent dual emissions, a schematic diagram of NIR luminescence and afterglow emissions of the Cr³⁺ ion in the RANTH is given in Figure 3. Processes I–IV represent the excitation and NIR emission processes related to the Cr³⁺ ion under UV light excitation. For the NIR afterglow emissions, both the conduction band transport mechanism (process V) and the tunnelling mechanism (process VI) simultaneously play key roles. The thermally stimulated luminescence (Figure S3) presents a superimposition of several peaks, suggesting the existence of shallow and deep trap levels. Other details have been discussed in our previous work. Because the ⁴T₂ level is slightly higher than the ²E level in the case of an intermediate crystal field as shown in Figure S2, the thermally populated electrons of the ²E state with an energy close to that at the crossover point can transfer back to the ⁴T₂ state. Thus, the thermal equilibrium between the ²E and ⁴T₂ states can be reached. Meanwhile, for surface Cr³⁺ ions, the weaker local crystal field strength will cause the energy gap between the bottoms of the ⁴T₂ and ²E parabolic states (ΔE₁) to be smaller than that of interior Cr³⁺ ions (ΔE₂). Under X-ray irradiation, massive photoelectrons and photoholes are formed in ZGGO and the energy transfer linked to Cr³⁺ ions occurs through a cascade process. The related luminescent processes are similar to those used during UV excitation.
To quantitatively describe the kinetic processes, we take the total numbers of populated electrons in the \(^{4}T_2\) and \(^{2}E\) states as \(N(^{4}T_2)\) and \(N(^{2}E)\), respectively. According to Boltzmann’s distribution, the relation of the populated electrons in the excited \(^{4}T_2\) and \(^{2}E\) states can be defined as in eq 1:

\[
N(^{4}T_2) = N(^{2}E) \times \exp(-\Delta E/k_BT)
\]

where \(T\), \(\Delta E\), and \(k_B\) are the absolute temperature, the energy gap between the bottoms of the \(^{4}T_2\) and \(^{2}E\) parabolas, and the Boltzmann constant, respectively. On the other hand, the integrated luminescence intensity (\(I\)) is proportional to the number of populated electrons (\(N\)), and their relationship can be expressed as \(I \propto N\). In theory, the intensity ratio (\(R\)) of the \(^{4}T_2 \rightarrow ^{4}A_2\) emission to the \(^{2}E \rightarrow ^{4}A_2\) emission can be expressed as eq 2:

\[
R = \frac{I(^{4}T_2)}{I(^{2}E)} = \frac{c(\nu_2)p_1^s g_2 h\nu_2 A_2}{c(\nu_1)p_1^s g_1 h\nu_1 A_1} \exp\left(-\frac{\Delta E}{k_BT}\right)
\]

\[
= C_0 \times \exp\left(-\frac{\Delta E}{k_BT}\right)
\]

The subscripts, 1 and 2, correspond to the \(^{2}E \rightarrow ^{4}A_2\) and \(^{4}T_2 \rightarrow ^{4}A_2\) transitions, respectively. The values of \(c(\nu_i)\) and \(c(\nu_j)\) are the responses of the detection systems. \(A_1\) and \(A_2\) are the branching ratios. \(p_1^s\) and \(p_2^s\) are the spontaneous radiative rates. \(g_1\) and \(g_2\) are the degeneracies. \(h\nu_1\) and \(h\nu_2\) are the corresponding photon energies. Here, for a small variation in temperature, \(C_0\) can be taken as a constant. Thus, the theoretical intensity ratios of the \(^{4}T_2 \rightarrow ^{4}A_2\) emissions of the surface and interior \(Cr^{3+}\) ions to the \(^{2}E \rightarrow ^{4}A_2\) emissions of the total contribution of the surface and interior \(Cr^{3+}\) ions, \(R_s'\) and \(R_i'\), respectively, can be expressed as eqs 3 and 4, respectively:

\[
R_s' = \frac{I_s'}{I_1 + I_2'} = C_s' \times \exp\left(-\frac{\Delta E_s}{k_BT}\right)
\]

\[
R_i' = \frac{I_i'}{I_1 + I_2'} = C_i' \times \exp\left(-\frac{\Delta E_i}{k_BT}\right)
\]

where \(C_s'\) and \(C_i'\) are also the prepositive constants. Relevant details are described in the Supporting Information.

3.3. Ratiometric Photoluminescence Thermometry. To experimentally employ the ratiometric photoluminescence thermometer, the temperature-dependent photoluminescence spectra of the ZGGO:Cr\(^{3+}\) NIR RANTH (\(\lambda_{\text{ex}} = 256\) nm) were recorded as shown in Figure 4a. All the spectra can be fitted by a sum of three Gaussian functions, and a typical fitting result of the photoluminescence spectrum at 310 K is shown in the inset of Figure 4a. In Figure 4a, it can be observed that the integrated intensity of the sharp emission at 696 nm (peak 2 and 3) around 712 nm increases with an increase in temperature from 298 to 325 K (in the physiological range\(^{2,5,7,38}\)). In Figure 4b, the temperature-dependent \(\ln(R_s')\) and \(\ln(R_i')\) related to surface and interior \(Cr^{3+}\) ions are given in Figure S4a. The obtained energy gaps between the \(^{4}T_2\) and \(^{2}E\) states of the surface (\(\Delta E_s\)) and interior \(Cr^{3+}\) ions (\(\Delta E_i\)) are 388 and 620 cm\(^{-1}\), respectively. Rai\(^{39}\) believed that \(\Delta E\) should be more than \(~200\) cm\(^{-1}\) to avoid a strong overlap, suggesting that temperature sensing in the
physiological temperature region based on the ratiometric photoluminescence strategy is possible.

To further unravel the mechanism of the ratiometric luminescence thermometer, the luminescence decays of the ZGGO:Cr\textsuperscript{3+} NIR RANTH monitored at 696 nm were acquired as shown in Figure 5. Each of the decay curves can be fitted by a four-exponential function, and the luminescence kinetic parameters related to the NIR emissions are summarized in Table 1. For each of the temperatures, the four obtained lifetimes (decay time on the order of milliseconds) and the two shorter components (decay time on the order of microseconds) are attributed to the spin-forbidden \( ^2E(\text{T}_2) \rightarrow ^4A_2 \) and spin-allowed \( ^4T_2(\text{E}) \rightarrow ^4A_2 \) transitions of Cr\textsuperscript{3+} ions, respectively. For the surface Cr\textsuperscript{3+} ions in nanoparticles, the lower symmetry of their occupied sites will lead to the lifetimes being shorter than those of the interior Cr\textsuperscript{3+} ions. The lifetimes, \( \tau_s \) and \( \tau_i \), related to the \( ^2E \rightarrow ^4A_2 \) transitions of the surface and interior Cr\textsuperscript{3+} ions show a tendency to decrease with an increase in the measured temperature, while those related to the \( ^4T_2 \rightarrow ^4A_2 \) transition exhibit the opposite behavior. This result suggests that more thermal population electrons in the \( ^4T_2 \) state are generated through the back-transfer from the \( ^2E \) state at higher temperatures.

3.4. Ratiometric Afterglow Thermometry. To demonstrate the ratiometric afterglow for temperature sensing, the temperature-dependent afterglow dual emissions of the ZGGO:Cr\textsuperscript{3+} NIR RANTH after UV irradiation were recorded as shown in Figure 6a. At various temperatures, the afterglow dual-emission spectra were measured 10 min after stopping a 5 min 256 nm irradiation. All the spectra can also be fitted by a sum of three Gaussian functions, and a typical fitting result of the afterglow emission spectrum measured at 310 K is shown in the inset of Figure 6a. The temperature-dependent \( \ln(R'_s) \) and \( \ln(R'_i) \) of the afterglow dual emissions of the RANTH are shown in Figure 6b. They also exhibit a linear relation with the reciprocal temperature as in Figure 6b. The corresponding temperature-dependent \( R'_s \) and \( R'_i \) and the related fitting results are shown in Figure S4b. The obtained \( \Delta E_s \) and \( \Delta E_i \) related to NIR ratiometric afterglow emissions are the same as those related to photoluminescence.

![Figure 5](image-url)

**Figure 5.** Temperature-dependent luminescence decay curves of the ZGGO:Cr\textsuperscript{3+} RANTH monitored at 696 nm upon 580 nm excitation. The red lines represent the fitting data.

<table>
<thead>
<tr>
<th>temp (K)</th>
<th>decay(^a) (( ^2E \rightarrow ^4A_2 )) (ms)</th>
<th>decay(^b) (( ^4T_2 \rightarrow ^4A_2 )) (( \mu s ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>( \tau_s = 2.15, \tau_i = 6.30 )</td>
<td>( \tau_s = 12.37, \tau_i = 146.52 )</td>
</tr>
<tr>
<td>301</td>
<td>( \tau_s = 2.11, \tau_i = 6.15 )</td>
<td>( \tau_s = 13.51, \tau_i = 225.30 )</td>
</tr>
<tr>
<td>304</td>
<td>( \tau_s = 2.08, \tau_i = 5.94 )</td>
<td>( \tau_s = 14.29, \tau_i = 229.58 )</td>
</tr>
<tr>
<td>307</td>
<td>( \tau_s = 2.02, \tau_i = 5.86 )</td>
<td>( \tau_s = 23.35, \tau_i = 257.06 )</td>
</tr>
<tr>
<td>310</td>
<td>( \tau_s = 2.01, \tau_i = 5.82 )</td>
<td>( \tau_s = 26.85, \tau_i = 262.60 )</td>
</tr>
<tr>
<td>313</td>
<td>( \tau_s = 1.96, \tau_i = 5.78 )</td>
<td>( \tau_s = 32.60, \tau_i = 266.60 )</td>
</tr>
<tr>
<td>316</td>
<td>( \tau_s = 1.92, \tau_i = 5.69 )</td>
<td>( \tau_s = 38.84, \tau_i = 350.17 )</td>
</tr>
<tr>
<td>319</td>
<td>( \tau_s = 1.87, \tau_i = 5.63 )</td>
<td>( \tau_s = 46.07, \tau_i = 363.21 )</td>
</tr>
<tr>
<td>322</td>
<td>( \tau_s = 1.83, \tau_i = 5.55 )</td>
<td>( \tau_s = 47.53, \tau_i = 388.68 )</td>
</tr>
<tr>
<td>325</td>
<td>( \tau_s = 1.77, \tau_i = 5.54 )</td>
<td>( \tau_s = 64.36, \tau_i = 391.47 )</td>
</tr>
</tbody>
</table>

\(^a\)Emission decay of the \( ^2E \rightarrow ^4A_2 \) transition. \( \tau_s \) and \( \tau_i \) represent the lifetimes related to the surface and interior Cr\textsuperscript{3+} ions, respectively. 

\(^b\)Emission decay of the \( ^4T_2 \rightarrow ^4A_2 \) transition.
To solidify this concept of time independence, the afterglow dual emissions were recorded at different delay times at various temperatures (301, 310, and 316 K) as shown in Figure 6c, Figure S5a, and Figure S5b, respectively. The higher time-stability features of $\ln(R_s')$ and $\ln(R_i')$ related to the surface and interior Cr$^{3+}$ ions are observed in panels d and e of Figure 6, respectively. These results indicate that the influence of the delay time on the accuracy and stability of the temperature sensing could be negligible. Therefore, this RANTH using NIR ZGGO:Cr$^{3+}$ superlong afterglow nanoparticles might be reliable.

### 3.5. Bifunctional Applications of Thermal Sensing for Simulated Blood and Bioimaging.

To demonstrate the usefulness of the ZGGO:Cr$^{3+}$ RANTH for temperature sensing and image tracing in the body, the ZGGO:Cr$^{3+}$ NIR superlong afterglow nanoparticles were dispersed in a HSA solution (5 mg/mL), and we applied a related RANTH/HSA solution to simultaneous NIR image tracing and real-time temperature sensing. The schematic of the related setup is shown in Figure 7a. After a 10 min UV light (256 nm) irradiation, the afterglow time of the RANTH/HSA solution was >1 h as shown in Figure 7b. The insets of Figure 7b are the digital photos under different light irradiances: white light, UV-lamp irradiation, and dark field for 30 s after the 15 min UV-lamp irradiation. Clear NIR imaging can be observed under a dark field, suggesting that image tracing of the blood circulation is likely. Meanwhile, the temperature-dependent NIR afterglow dual emissions of the RANTH/HSA solution at different pH values (7, 7.5, and 8) could be obtained as shown in Figure 7c, Figure S6, and Figure S7, respectively. At different pH values, the $\ln(R_s')$ and $\ln(R_i')$ values related to surface and interior Cr$^{3+}$ ions as a function of reciprocal temperature are given in the insets of Figure 7c and Figures S6 and S7. For interior Cr$^{3+}$ ions, nearly the similar temperature-dependent $\ln(R_i')$ and energy gaps ($\Delta E_i \approx 620 \text{ cm}^{-1}$) were acquired at different pH values. However, the $\ln(R_s')$ values related to surface Cr$^{3+}$ ions show a pH-dependent behavior. These results suggest that the NIR RANTH using interior Cr$^{3+}$ ions is a more appropriate way to perform simultaneous temperature sensing and image tracing in a complex body environment compared to using surface Cr$^{3+}$ ions.

To further reveal the temperature response of the RANTH in the HSA solution, the thermal sensitivity ($S$) is defined by eq 7:
The $R'_s$ and $R'_i$ values related to the surface and interior Cr$^{3+}$ ions, respectively, at various temperatures are shown in Figure S8 (pH 7). On the basis of the fitting data shown in Figure S8, the obtained thermal sensitivity curves of the ZGGO-Cr$^{3+}$ RANTH in the HSA solution using the surface and interior Cr$^{3+}$ ions are shown in Figure 7d. Their thermal sensitivities are in the range of 0.026−0.028 and 0.043−0.047 K$^{-1}$, respectively, in the
physiological temperature region (298–325 K). Although the thermal sensitivities related to the surface Cr\(^{3+}\) ions are lower than those related to the interior Cr\(^{3+}\) ions, both of them are \(\sim 1\) order of magnitude higher than that using the upconversion luminescence method,\(^\text{7,15,16}\) The higher sensitivities may be related to the larger \(R_i'\) and \(R_s'\) due to the spontaneous radiative rate of the \(^4T_2 \rightarrow ^2A_2\) transition being higher than that of the \(^4E \rightarrow ^2A_2\) transition. In particular, the lifetimes of the \(^4T_2 \rightarrow ^2A_2\) and \(^4E \rightarrow ^2A_2\) emissions of the interior Cr\(^{3+}\) ions are in the range of 100–400 \(\mu s\) and 5–7 ms,\(^\text{40–43}\) respectively, as shown in Table 1. Thus, the spontaneous radiative rate ratios \((p_i'/p_i)\) of the luminescent centers at various temperatures are in the range of 15–50, which are 1 order of magnitude higher than those \((\sim 1)\) in the case of upconverted luminescence. Therefore, from eqs 3, 4, and 7, our larger pre-exponential factors \(C_i'\) and \(C_s'\) lead to the larger \(R_i'\) and \(S\) values.

To perform temperature sensing using the ZGGO:Cr\(^{3+}\) RANTH at any time, an X-ray re-irradiation strategy for intensifying the undetectable afterglow emission intensity (the yellow line) was employed as shown in Figure 7e. After two X-ray re-irradiations, temperature sensing in the ranges of 298–310 and 313–325 K was re-performed. Meanwhile, for the ZGGO:Cr\(^{3+}\) RANTH using the interior Cr\(^{3+}\) ions, a nearly similar energy gap \((\Delta E \approx 620 \text{ cm}^{-1})\) and a nearly similar thermal similar sensitivity \((0.043–0.047 \text{ K}^{-1})\) were obtained as shown in the inset of Figure 7e and Figure S9. These results suggest that the influence of X-ray re-irradiation on the accuracy and stability of thermal sensing could be negligible.

To demonstrate \textit{in vivo} bioimaging using the ZGGO:Cr\(^{3+}\) RANTH, a 200 \(\mu\)L solution containing ZGGO:Cr\(^{3+}\) nanoparticles (10 mg/mL) was administered to a C57BL/6 mouse through a subcutaneous injection. Before the injection, the solution was irradiated with a 254 nm UV lamp for 5 min. The clear NIR afterglow signals of the RANTH existing in the injected area of the mouse were observed when the detecting time was increased from 10 min to 3 h, as shown in Figure 8 (exposure time, 15 s). When the exposure time was set to 120 s, the central afterglow intensity of the image acquired 3 h after the injection, as shown in Figure S10, was 7 times stronger than that with an exposure time of 15 s. Compared with the data reported by Abdulkayum et al., we can deduce that the \textit{in vivo} bioimaging using the ZGGO:Cr\(^{3+}\) RANTH can last \(\sim 10\) h. Although there was obvious toxicity when cells ingested excess heavy metal ions (e.g., Zn\(^{2+}\) and Cr\(^{3+}\)),\(^\text{44–46}\) it was found that the improved biocompatibility and decreased biotoxicity of ZGGO:Cr\(^{3+}\) nanoparticles can be reached through surface modification by silica as reported by Abdulkayum et al.\(^\text{39}\) To reveal the existence of silica-coated ZGGO:Cr\(^{3+}\) (ZGGO:Cr\(^{3+}\)@SiO\(_2\)) nanoparticles, a TEM image of ZGGO:Cr\(^{3+}\)@SiO\(_2\) nanocomposites and a high-resolution TEM image of the marked area are shown in panels a and b of Figure S11, respectively. It can be found that a thin \((4 \text{ nm})\) silica layer coated on the surface of a ZGGO:Cr\(^{3+}\) particle appears, which is proven by the presence of the EDS peak associated with the Si atom, as shown in Figure S11c. The slight aggregation behavior of ZGGO:Cr\(^{3+}\)@SiO\(_2\) nanoparticles might be related to the increased number of isolated silanol groups and the decreased hydration force caused by the thin silica layer.\(^\text{47}\) To evaluate the biocompatibility of ZGGO:Cr\(^{3+}\)@SiO\(_2\) nanocomposites, the MTT assays were performed on L929 cell lines as shown in Figure S12a. Compared with those of the control group, the cell viabilities show a decrease from 100 to 65% as the concentration of ZGGO:Cr\(^{3+}\) increases from 0 to 100 \(\mu g/mL\). After silica coating, the cell viabilities are increased to \(>80\%\) in the concentration range of 12.5–100 \(\mu g/mL\). In particular, in the case of 12.5 \(\mu g/mL\), the improved biocompatibility of ZGGO:Cr\(^{3+}\) nanoparticles after silica coating can be clearly observed, as shown in Figure S12b–d. Thus, it is possible for the ZGGO:Cr\(^{3+}\) RANTH to have potential applications in biomedicine.\(^\text{28}\)

### 3.6. Effect of the Tissue Penetration Depth on Temperature Sensing

To prove the applicability of deep tissue temperature sensing, a pork tissue slice of varying thickness \((0–15 \text{ mm})\) was placed between the ZGGO:Cr\(^{3+}\) RANTH and the detector. The related experimental setup is shown in Figure 9a. The tissue depth-dependent NIR afterglow dual-emission spectra of the ZGGO:Cr\(^{3+}\) RANTHs were recorded at 298 K as shown in Figure 9b. The penetration depth of the NIR afterglow emissions could reach \(>15\) mm. For various pork tissue depths, the \(\ln(R_i')\) and \(\ln(R_s')\) values related to the surface and interior Cr\(^{3+}\) ions, respectively, are shown in Figure 9c. A typical fitting result in the case of a 3 mm tissue depth is shown in the inset of Figure 9c. The \(\ln(R_i')\) values derived from the interior Cr\(^{3+}\) ions almost do not rely on tissue depth, while \(\ln(R_s')\) values related to the surface Cr\(^{3+}\) ions tend to increase. It might be related to the longer-wavelength emission of the \(^4T_2(4F) \rightarrow ^2A_2\) transition from the surface ions relative to the interior ions, and this leads to the slower attenuation of NIR light.\(^\text{48}\)

To further demonstrate the temperature sensing at a certain tissue depth, the temperature-dependent NIR afterglow
emissions of the ZGGO:Cr³⁺ RANTH were obtained in the case of a 3 mm tissue thickness, as shown in Figure 9d. For each of the spectra measured at different temperatures, it can be fitted by three Gaussian functions, and a typical fitting result (at 310 K) is shown in panel d.
shown in the inset of Figure 9d. The obtained $\ln(R_i')$ and $\ln(R_s')$ values related to the interior and surface Cr$^{3+}$ ions, respectively, as a function of reciprocal temperature are given in Figure 9e. It can be found that the $\ln(R_i')$ value has a better linear relation with the reciprocal temperature than the $\ln(R_s')$ value does. In the cases of 1 and 5 mm thick tissue, similar behaviors can be observed, as shown in Figure S13. These results further suggest that the ZGGO:Cr$^{3+}$ RANTH using the interior Cr$^{3+}$ ions is more suitable for temperature sensing. In addition, for pork tissues with different thicknesses (3, 5, and 10 mm), NIR afterglow emissions of the RANTH covered by them lasted for >4 h as shown in Figure 9f. Because most newborn muscle is almost equal to or smaller than 10 mm, 49,50 it is likely that the ZGGO:Cr$^{3+}$ RANTH mentioned above is applied to temperature sensing and real-time tracing.

Although the afterglow intensity of ZGGO:Cr$^{3+}$ nanoparticles can be intensified through an X-ray re-irradiation strategy, the excessive dose of X-ray radiation will injure tissues and organs in vivo. To overcome the aforementioned drawback, a NIR light re-excitation strategy was performed when the NIR afterglow intensity of the ZGGO:Cr$^{3+}$ RANTH was undetectable after a long-term detection. Here, an 808 nm laser was used as a re-excitation source because of its high level of penetration as reported by He et al.51 The photostimulatable luminescence spectrum of the ZGGO:Cr$^{3+}$ RANTH covered by a 3 mm thick pork tissue under 808 nm excitation (200 mW/cm$^2$) with a 30 min delay time after stopping UV irradiation is shown in Figure 10a. It can be found that the intense NIR emission intensity ($\lambda_{em} = 696$ nm) can be acquired when the 808 nm laser is on (duration time, 30 s). The intense NIR emissions can be ascribed to the contribution of deeper traps due to the 808 nm stimulation. When the 808 nm laser is off, a relatively slow decay appears (duration time, 5 min). In particular, the afterglow intensity of the ZGGO:Cr$^{3+}$ RANTH after the 808 nm stimulation is 40 times stronger than that before the 808 nm stimulation (background). The intensified afterglow intensity originates from the contribution of the shallower traps, which capture the electrons released by the deeper traps during the 808 nm stimulation.52 In addition, it is found that human skin is not photodamaged when the 808 nm laser intensity is lower than $\sim 330$ mW/cm$^2$.53,54 In our case, the lower intensity of NIR irradiation (200 mW/cm$^2$) further suggests that the ZGGO:Cr$^{3+}$ RANTH is suitable for simultaneous in situ in vivo bioimaging and temperature sensing. Figure 10b shows the afterglow emission spectra of the ZGGO:Cr$^{3+}$ RANTH covered by a 3 mm thick pork tissue measured at different temperatures (temperature range of 298–325 K). During detection, the ZGGO:Cr$^{3+}$ RANTH was totally re-excited four times; i.e., the 808 nm re-excitations were separately performed at 298, 313, 319, and 325 K, and each of them lasted 30 s. It can be found that the afterglow intensity decreases with an increase in the number of excitations. This is related to the depletion of the captured electrons in deeper traps caused by the previous re-excitations. The $\ln(R_i')$ value is also perfectly linearly related to the reciprocal temperature, as shown in the inset of Figure 10b. It suggests that the multiple re-excitations do not influence the accuracy of the temperature sensing.

4. CONCLUSION

In this report, a RANTH with high reliability has been demonstrated by using a NIR superlong afterglow nanoparticle (ZGGO:Cr$^{3+}$) strategy. Herein, the potential bifunctions (temperature sensing and NIR in vivo bioimaging) were realized on the basis of NIR afterglow dual emissions of the surface and interior Cr$^{3+}$ ions. Meanwhile, the cases of temperature sensing related to the surface or interior Cr$^{3+}$ ions were separated and discussed clearly on the basis of the d electron configuration of the Cr$^{3+}$ ion,51 which is sensitive to the local crystal field environment. In particular, the RANTH related to the NIR afterglow dual emissions of the interior Cr$^{3+}$ ions exhibited pH-independent and time-stability features, which can dramatically weaken the possible influence derived from the subsequent bifunctional scenarios. Strikingly, in the case of deep tissue temperature sensing, the NIR RANTH can work even though the tissue depth reaches 15 mm, and similar detection sensitivities can be acquired for various tissue thicknesses. Significantly, a highly sensitive temperature sensing (0.043–0.047 K$^{-1}$) was demonstrated in the physiological temperature
range (298−325 K), which is ∼1 order of magnitude higher than that using an upconversion luminescence strategy. This is related to the larger spontaneous radiative rate ratios (15−50) of the dual emissions of Cr³⁺ ions at various temperatures, which are 1 order of magnitude higher than those (~1) in the case of upconverted luminescence. Therefore, the NIR RANTH with a high thermal sensitivity could produce a more precise temperature reading, which has potential applications in controlling the small temperature variation in the physiological temperature range during diagnosis and therapy. 35−37 Meanwhile, temperature sensing could be re-performed by multiple re-excitations using an 808 nm laser, and similar detection sensitivities can be obtained. Thus, the optical multiplexing capability of this novel bifunctional ZGGO:Cr³⁺ RANTH is expected to further develop for the future demands in the life sciences.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemmater.7b01958.

Additional analysis data, size distribution, TSL NIR afterglow curve, and theoretical model description (PDF)

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**Notes**

The authors declare no competing financial interest.

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