Supplementary Information

Core-shell hybrid upconversion nanoparticles carrying stable nitroxide radicals as potential multifunctional nanoprobes for upconversion luminescence and magnetic resonance dual-modality imaging

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Fig. S1 FTIR spectra of (a) 3-aminopropyltriethoxysilane (APTS), (b) 4-Carboxyl TEMPO radicals and (c) the silanizing TEMPO radical precursors. The spectrum of silanizing TEMPO radical precursors (c) show the presence of characteristic bands at 1643 cm\(^{-1}\) corresponding to the stretching vibration of the C=O groups (amide I). The bands at 1549 cm\(^{-1}\) are assigned to the N–H groups (amide II). The bands at 2936 and 2975 cm\(^{-1}\) are attributed to the stretching vibrations of –CH\(_2\)– and –CH\(_3\) groups of the ethyl chains, respectively. Compared with 3-aminopropyltriethoxysilane, the spectrum of silanizing TEMPO radical precursors (c) also shows the presence of two characteristic bands at 1075 and 800 cm\(^{-1}\) assigned to the Si–O–Et groups. Moreover, it is worth noting that this successful amidation reaction was also revealed by the disappearance of stretching vibration of C=O groups at 1731 and 1690 cm\(^{-1}\) of the 4-Carboxyl TEMPO radicals.
Fig. S2 ESI-MS spectrum of the silanizing nitroxide radical precursor. (MS $m/z$: [M$^+\$] calcd for $C_{19}H_{39}N_2O_5Si$, 403.61; found [M + Na]$^+$: 426.2.

Fig. S3 TEM image of TEMPO@SiO$_2$ nanoparticles.
Fig. S4 The ESR spectra recorded in aqueous solution of (a) silanizing TEMPO radical precursors, (b) TEMPO@SiO$_2$ nanoparticles, (c) supernatant after three cycles centrifugal separation of TEMPO@SiO$_2$ nanoparticles precipitate, (d) SiO$_2$ nanoparticles.
Fig. S5 Schematic illustration of the paramagnetic SiO₂ network formation.
Fig. S6 Low magnification TEM images of the core and core-shell UCNPs shown in Fig. 3. These results demonstrate the 2D assembly of UCNPs, confirming the high monodispersity and uniformity of the obtained core and core-shell UCNPs with different layers of NaYF$_4$ shell. All scale bar are 200 nm.
**Fig. S7** Size distribution of core NaYF₄:Yb,Er and core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs with different layers of NaYF₄ shell. The length of the obtained UCNPs increased from 19.6 ± 1.1 nm to 23.3 ± 0.8 nm, 25.5 ± 1.1 nm, 27.2 ± 1.1 nm, 29.3 ± 1.0 nm, 31.2 ± 1.1 nm and 33.4 ± 1.2 nm after growing different thickness of NaYF₄ shells on the core NaYF₄:Yb,Er, and the width increased from 19.6 ± 1.1 nm to 20.2 ± 0.8 nm, 21.1 ± 0.9 nm, 21.7 ± 0.9 nm, 23.1 ± 1.0 nm, 24.1 ± 0.9 nm and 25.1 ± 1.1 nm after growing different layers of NaYF₄ shells on the core NaYF₄:Yb,Er.
Fig. S8 Schematic of anisotropic shell growth of NaYF$_4$ shell on the core NaYF$_4$::Yb,Er. Because $\beta$-NaYF$_4$ UCNP has a highly anisotropic structure, the nanoparticles preferentially grow into the ellipsoidal shape.$^{1, 2}$

Fig. S9 XRD patterns of the core NaYF$_4$::Yb,Er (a) and core-shell NaYF$_4$::Yb,Er/NaYF$_4$ UCNP with 15 (b) and 30 (c) layers of NaYF$_4$ shell. Calculated line pattern for hexagonal phase NaYF$_4$ (bottom plot) is shown for reference (black). All diffraction peaks of these nanoparticles could be clearly indexed to hexagonal phase NaYF$_4$ crystals.
Fig. S10 Selected-area electron diffraction (SAED) pattern of core-shell NaYF$_4$:Yb,Er/NaYF$_4$ UCNPs with 30 layers of NaYF$_4$ shell. This SAED pattern demonstrates a perfect hexagonal crystal structure of the resulted core-shell NaYF$_4$:Yb,Er/NaYF$_4$ UCNPs with 30 layers of NaYF$_4$ shell. The pattern shows clear polycrystalline diffraction rings corresponding to the (100), (110), (111), (201), (311), and (321) planes of hexagonal phase NaYF$_4$ lattice.
Fig. S11 The enhancement times of total integrated upconversion emission intensity from 350 nm to 700 nm of core-shell UCNPs versus the corresponding layers of inner NaYF$_4$ shell.

Fig. S12 XPS spectrum of UCNP@TEMPO@SiO$_2$ nanocomposites.
**Fig. S13** EDX analysis of UCNP@TEMPO@SiO$_2$ nanocomposites.

**Fig. S14** DLS size distribution of the UCNP@TEMPO@SiO$_2$ nanocomposites synthesized with variable amounts of silanizing TEMPO radical precursors added during the process of copolymerization.
Fig. S15 Zeta potential of (a) UCNP@TEMPO@SiO$_2$, (b) UCNP@TEMPO@SiO$_2$-NH$_2$, and (c) PEGylated UCNP@TEMPO@SiO$_2$. (Performed in 1 mM pH 7.4 Tris-HCl buffer solution)
Fig. S16 Inverted fluorescence microscope images of HeLa cells incubated with PEGylated UCNP@TEMPO@SiO$_2$ nanocomposites at different concentrations (0, 40, 80, 120, 160, 200 $\mu$g/mL) for 6 h. Each group can be classified to the DIC image, nuclei of cells (being dyed by Hoechst 33258 for visualization), upconversion luminescence image (UCL) and the overlay of the three above, respectively.
Fig. S17 UV-vis spectra of PEGylated UCNP@TEMPO@SiO$_2$ nanocomposites (15 mg/mL) in MeOH/H$_2$O (1:1) solution (red line), the PEGylated UCNP@SiO$_2$ without the TEMPO radicals doping (15 mg/mL) were used as reference (black line). Photograph (a) shows the transparency of the PEGylated UCNP@TEMPO@SiO$_2$ nanocomposites (15 mg/mL) in MeOH/H$_2$O (1:1) solution. Photograph (b) and (c) show the strong upconversion emission of this solution in the daylight and in the dark under excitation of 980 nm laser (power 0.6 W), respectively.
**Geometric structure analysis of core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs**

According to the TEM images, the average diameter of core NaYF₄:Yb,Er UCNPs is 19.6 ± 1.1 nm, the average length and width of rod-like core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs with 30 layers of NaYF₄ are 33.4 ± 1.2 nm and 25.1 ± 1.1 nm, respectively. So the approximative volume ratio of NaYF₄:Yb,Er core and NaYF₄ shell is calculated by following formula:

\[
\frac{V_{\text{core}}}{V_{\text{shell}}} = \frac{\frac{4}{3} \pi \left( \frac{d}{2} \right)^3}{\pi \left( \frac{D}{2} \right)^2 \times H – \frac{4}{3} \pi \left( \frac{d}{2} \right)^3} = 1:3.19
\]

This value is approximately equal to the molar ratio of the amount of lanthanide input for fabricate core and shell (1:3) respectively.

**Calculation of TEMPO radicals loading in core-shell UCNP@TEMPO@SiO₂ nanocomposites (molar TEMPO:UCNP ratio)**

The amount of TEMPO radicals conjugated in the outer SiO₂ shell of NaYF₄:Yb,Er/NaYF₄ UCNPs is estimated using the following procedures:

- **A hexagonal phased (β) NaYF₄ unit cell has the following parameters:** \(a=0.596 \text{ nm, } c=0.353 \text{ nm}\). Since \(Y^{3+} (0.0880 \text{ nm), } Yb^{3+} (0.0859 \text{ nm), } Er^{3+} (0.0881 \text{ nm})\) have a quite similar ion radius, the dopant induced lattices distortions in hexagonal phased (β) NaYF₄ could be negligible. The UCNPs had monodisperse size distribution and uniform geometric structure. The average diameter of sphere-like NaYF₄:Yb,Er core UCNPs is 19.6 ± 1.1 nm (d), the average length and width of rod-like core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs are 33.4 ± 1.2 nm (H) and 25.1 ± 1.1 nm (D), respectively. Based on these preconditions, we calculate the “molecular weight” of NaYF₄:Yb,Er/NaYF₄ core-shell UCNPs firstly.

**Volume of NaYF₄:Yb,Er core UCNPs unit cell** (Na₁₅Y₁₁F₆₀, \(a=0.596 \text{ nm, } b=0.353 \text{ nm}\))

\[
V_{\text{cell}} = \frac{3}{2} \times a^2 = 0.1086 \text{ nm}^3
\]

**Volume of core and shell** (d= 19.6 ± 1.1 nm, D= 25.1 ± 1.1 nm, H= 33.4 ± 1.2 nm)

\[
V_{\text{core}} = \frac{4}{3} \pi \left( \frac{d}{2} \right)^3 = 3942 \text{ nm}^3
\]

\[
V_{\text{shell}} = \pi \left( \frac{D}{2} \right)^2 \times H – \frac{4}{3} \pi \left( \frac{d}{2} \right)^3 = 12585 \text{ nm}^3
\]

The number of unit cell in core and shell

\[
N_{\text{core}} = 1.5 \times V_{\text{core}} / V_{\text{cell}} = 5.4 \times 10^4 \quad N_{\text{shell}} = 1.5 \times V_{\text{shell}} / V_{\text{cell}} = 1.2 \times 10^5
\]
The “molecular weight” of NaYF₄:18%Yb,2%Er/NaYF₄ core-shell UCNPs

\[ M_{\text{UCNPs}} = N_{\text{core}} \times (M_{\text{Na}} + 80\% M_{\gamma} + 18\% M_{\text{Yb}} + 2\% M_{\text{Er}} + 4 \cdot M_F) + N_{\text{shell}} \times (M_{\text{Na}} + M_{\gamma} + 4 \cdot M_F) \]

\[ = N_{\text{core}} \times 204.6 \text{ g/mol} + N_{\text{shell}} \times 187.9 \text{ g/mol} = 3.36 \times 10^7 \text{ g/mol} \]

The absorption of PEGylated UCNP@TEMPO@SiO₂ nanocomposites (15 mg/mL) at 435 nm in MeOH/H₂O (1:1) solution was 0.112. According to Beer–Lambert law (\(A = \varepsilon \times b \times c\)) and the molar absorptivity coefficient (\(\varepsilon\)) of TEMPO radical (13.3 dm³ mol⁻¹ cm⁻¹), the concentration of TEMPO radicals was calculated to 8.4 mM (1.7 mg/mL). Furthermore, the concentration of Y element in this solution was 3.5 mg/mL measured by ICP-AES. In comparison with core NaYF₄:Yb,Er UCNPs, the doping amounts of Yb and Er in the core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs with 30 layers of NaYF₄ is negligibly small. Thus, the concentration of core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs (Y % = 47%) was calculated to 3.5/0.47 = 7.5 mg/mL.

Numbers of core-shell NaYF₄:Yb,Er/NaYF₄ UCNPs in the MeOH/H₂O (1:1) solution of UCNP@TEMPO@SiO₂ nanocomposites (15 mg/mL):

\[ N_{\text{UCNPs}} = \frac{7.5 \times 10^{-3}}{M_{\text{UCNPs}}} \]

Numbers of TEMPO radicals in the MeOH/H₂O (1:1) solution of UCNP@TEMPO@SiO₂ nanocomposites (15 mg/mL):

\[ N_{\text{TEMPO}} = \frac{1.7 \times 10^{-3}}{M_{\text{TEMPO}}} \]

The average numbers of TEMPO radicals embedded in the compact SiO₂ shell coated on the surface of per UCNP:

\[ \frac{N_{\text{TEMPO}}}{N_{\text{UCNPs}}} = \frac{1.7}{7.5} = 3.8 \times 10^4 \]

References in Supplementary Information