

Tunable power-dependent upconversion emission of co-doped Er^{3+} – Tm^{3+} nanoparticles for bioimaging

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Abstract. Lanthanide-doped upconversion nanoparticles (UCNPs) have emerged as promising platforms for bioimaging and theranostics owing to their unique ability to convert near-infrared (NIR) excitation into visible emission. Controlling their emission behavior is critical for both mechanistic understanding and biomedical translation. In this work, we synthesized NaYF_4 : Yb^{3+} , Er^{3+} , Tm^{3+} UCNPs via a hydrothermal route and systematically investigated their power-dependent luminescence. Log–log slope analysis clarified photon participation in different emission bands, revealed competition between $\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$ and $\text{Yb}^{3+} \rightarrow \text{Tm}^{3+}$ pathways, and identified saturation effects at higher excitation power. Based on these insights, the nanoparticles were functionalized with silica–TPGS to improve colloidal stability, dispersibility, and biocompatibility. Preliminary biological evaluation with MCF-7 breast cancer cells demonstrated efficient uptake, bright intracellular luminescence, and dose-dependent cytotoxicity ($\text{GI}_{50} = 0.26 \pm 0.02$ ppm). These findings highlight excitation-power control as a powerful strategy for tailoring UCNP emission properties, laying the foundation for advanced applications in ratiometric sensing, multicolor imaging, and cancer theranostics.

Keywords: upconversion; nanoparticles; power; NaYF_4 : Yb^{3+} , Er^{3+} , Tm^{3+} ; bioimaging.

Classification numbers: 78.55.-m; 78.60.-b; 87.85.Pq; 87.85.Qr.

1. Introduction

Upconversion nanoparticles (UCNPs) based on lanthanide-doped fluorides have attracted significant interest for biomedical applications due to their unique optical properties. Unlike conventional fluorophores, UCNPs can convert near-infrared (NIR) excitation into visible emission, offering several distinct advantages. First, NIR excitation largely avoids overlapping with endogenous biomolecules, resulting in minimal tissue autofluorescence and greatly improved signal-to-background contrast. Second, lanthanide-doped UCNPs exhibit high photostability due to their shielded 4f–4f electronic transitions, resisting photobleaching even under prolonged irradiation. Third, the use of low-energy NIR light reduces photodamage and photothermal effects in biological samples, thereby preserving cell viability and enabling long-term imaging studies. These advantages clearly distinguish UCNPs from conventional organic dyes and quantum dots, underscoring their suitability for advanced biomedical imaging and sensing [1–13].

Among various host–dopant systems, β - NaYF_4 doped with Yb^{3+} as sensitizer and $\text{Er}^{3+}/\text{Tm}^{3+}$ as activators is particularly attractive because it enables simultaneous green, red, and blue emissions. Such multicolor emission can be exploited for multiplexed imaging and intracellular sensing, while also providing opportunities for designing theranostic platforms that combine imaging and therapy [14–16].

A crucial factor in advancing UCNPs toward biomedical use is controlling their emission behavior. Previous studies have mainly focused on compositional tuning (dopant concentration, core–shell engineering) to adjust luminescence [17–25]. However, excitation power remains a relatively underexplored parameter that can provide both mechanistic insights and practical tunability. Power-dependent studies allow researchers to identify the number of photons involved in each emission process and reveal the competition between $\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$ and $\text{Yb}^{3+} \rightarrow \text{Tm}^{3+}$ channels, enabling in situ modulation of emission color without altering nanoparticle composition [26–32].

Unlike previous studies that primarily rely on modifying dopant composition or core–shell architectures to tune upconversion emission, this study demonstrates that excitation power alone can serve as a practical, composition-independent parameter for real-time spectral modulation. Moreover, by integrating power-controlled optical behavior with silica–TPGS surface engineering and intracellular imaging, we establish a direct connection between fundamental photophysical mechanisms and biological applicability, presenting a convenient and versatile strategy for optimizing imaging contrast without altering nanoparticle composition.

For biomedical translation, surface modification is equally essential. Silica coating improves stability and prevents direct ion leakage, while TPGS functionalization enhances aqueous dispersibility, promotes cellular uptake, and provides additional biocompatibility. These strategies make UCNPs promising candidates for diagnostic and therapeutic applications [33–35].

In this study, we first investigate the power-dependent luminescence of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs to clarify photon participation, pathway competition, and saturation effects. Based on these mechanistic insights, the nanoparticles are subsequently functionalized with silica–TPGS to improve stability and biocompatibility. Finally, a preliminary biological test with MCF-7 breast cancer cells demonstrates efficient uptake, bright intracellular luminescence, and dose-dependent cytotoxicity. While the cellular application is only introduced briefly, it illustrates the potential of linking fundamental slope analysis with surface engineering to guide the design of multifunctional UCNPs for future biomedical use.

2. Experiment

2.1. Chemicals

All chemicals were of analytical grade and used as received without further purification. Yttrium nitrate hexahydrate [$\text{Y}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$], ytterbium nitrate pentahydrate [$\text{Yb}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$], erbium nitrate pentahydrate [$\text{Er}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$], and thulium nitrate pentahydrate [$\text{Tm}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$] (all 99.9%) were used as precursors. Sodium fluoride (NaF, 99.5%), ammonium hydroxide solution (25%), tetraethyl orthosilicate (TEOS, 99%), ethylene glycol (99.95%), ethanol (99.8%), isopropyl alcohol (99%), d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS), cyclohexane and sodium hydroxide (NaOH, 99.0%), oleic acid $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ (92%) were purchased from Sigma-Aldrich, Merck and Fisher Scientific. Deionized water was used throughout all experiments.

2.2. Synthesis of $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs

The upconversion nanoparticles were synthesized via a hydrothermal method (Fig. 1) [31, 32]. The precursor ratio was $\text{Y}^{3+}:\text{Yb}^{3+}:\text{Er}^{3+}:\text{Tm}^{3+} = 79:19:20:1:1$. In a typical preparation, 6 mL NaOH solution, 30 mL ethanol, 30 mL ethylene glycol, oleic acid and stoichiometric amounts of $\text{Y}(\text{NO}_3)_3$ (31.6 mL), $\text{Yb}(\text{NO}_3)_3$ (7.6 mL), $\text{Er}(\text{NO}_3)_3$ (4 mL), and $\text{Tm}(\text{NO}_3)_3$ (4 mL) were mixed under magnetic stirring for 15 min. Subsequently, 12 mL of NaF solution was added dropwise to the mixture, which was further stirred for 6 h at 150°C.

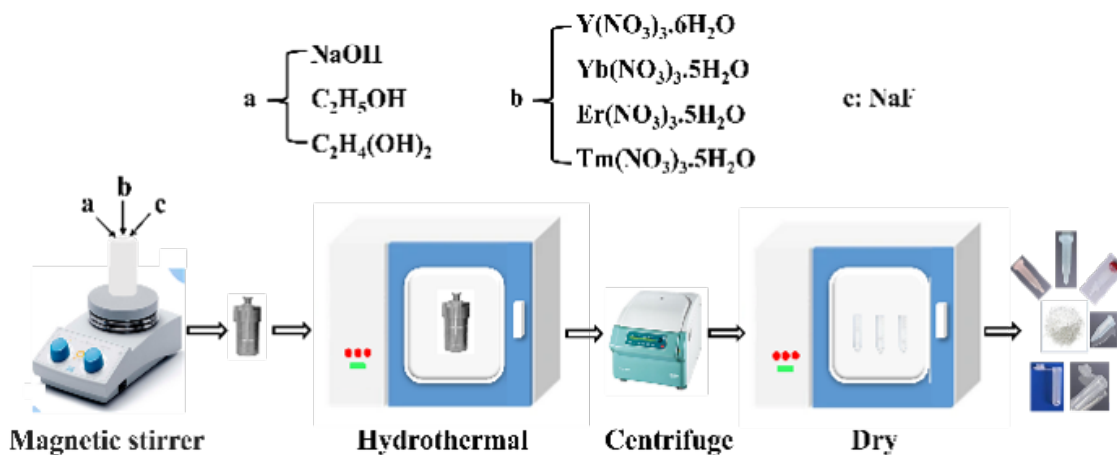


Fig. 1. Schematic illustration of the hydrothermal synthesis of $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs.

The precursor solution was transferred into a Teflon-lined stainless-steel autoclave and heated at 180°C for 24 h. After cooling to room temperature, the products were collected by centrifugation, washed repeatedly with ethanol and water, and dried at 60°C for 24 h to yield β - $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs as a fine powder [27, 28]. Yb^{3+} , Er^{3+} , and Tm^{3+} ions replace Y^{3+} sites in the NaYF_4 lattice due to their similar ionic radii and identical charge state, enabling isovalent substitution.

2.3. Surface modification

For biological applications, the UCNPs were coated with silica using a sol–gel process. Briefly, the nanoparticles were dispersed in ethanol, followed by the addition of TEOS, ammonium hydroxide, and water under continuous stirring for 12 h. The silica-coated UCNPs were subsequently functionalized with TPGS ligands. The procedure was as follows: 100 mg TPGS was dispersed in 10 ml of cyclohexane and activated at room temperature for 1 hour. 200 mg the $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ @silica nanoparticles was dispersed in 10 ml ethanol and then added dropwise in the activated TPGS solution and stirred for 60 minutes by magnetic stirrer (solution 1). Then, 20 ml of deionized water was added to solution 1 and it was kept stirring at 70 °C for 60 minutes to evaporate the cyclohexane. $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ @silica-TPGS biofunctionalized nanoparticles could be obtained by centrifugation.

2.4. Cell culture and labeling

MCF-7 human breast cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells at ~70–80% confluency were harvested and incubated with $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ @silica-TPGS biofunctionalized nanoparticles (2% dispersion) for 3 h at 37 °C. After incubation, cells were washed with phosphate-buffered saline (PBS) to remove unbound particles. The cells were then fixed with 4% formaldehyde for 30 min, washed again with PBS, and stained with Hoechst 33342 (2 µg/mL) for 15 min in dark.

2.5. Cytotoxicity assay

The cytotoxicity of the $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ @silica-TPGS biofunctionalized nanoparticles was evaluated using the sulforhodamine B (SRB) assay. MCF-7 cells were pre-seeded in 96-well plates for 24 h and then treated with varying concentrations of the nanoparticles for an additional 24 h. Ellipticine was used as a positive control.

After treatment, cell viability was quantified based on absorbance measurements and expressed relative to untreated control cells. Cytotoxicity assays were conducted using at least three independent biological replicates, each performed in triplicate. GI_{50} values were calculated using TableCurve 2D v4 software.

2.6. Characterization techniques

The structural of the UCNPs were characterized using a combination of complementary techniques. X-ray diffraction (XRD, Siemens D5000, Cu $K\alpha$ radiation, $\lambda = 1.5406 \text{ \AA}$, $10^\circ \leq 2\theta \leq 80^\circ$) was employed to determine the crystallographic phases and confirm the formation of the hexagonal β - NaYF_4 structure. The morphology of the UCNPs were examined by field-emission scanning electron microscopy (FE-SEM).

Optical properties were evaluated by recording upconversion emission spectra on an IHR 550 spectrometer under continuous-wave 980 nm laser excitation. For power-dependent measurements, excitation powers ranging from 300 to 600 mW were applied, with values carefully calibrated at the sample position using a powermeter.

Spectra were recorded under constant detector settings and fixed acquisition time; power values correspond to the sample plane as calibrated by a powermeter.

The biological performance of the UCNPs was assessed through fluorescence microscopy imaging using a Zeiss inverted fluorescence microscope to visualize cellular uptake in MCF-7

breast cancer cells. Finally, cytotoxicity was evaluated by sulforhodamine B (SRB) assays, with ellipticine included as a positive control to benchmark cell growth inhibition.

3. Results and discussion

3.1. Structural characterization and morphology

The crystalline structure of the synthesized $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ nanoparticles was first examined by X-ray diffraction (XRD). As shown in Fig. 2, sharp and intense diffraction peaks are observed at $2\theta \approx 17.2^\circ, 30^\circ, 31.9^\circ, 34.7^\circ, 43.6^\circ, 47.1^\circ, 53.9^\circ$, and 62.5° , which can be indexed to the hexagonal $\beta\text{-NaYF}_4$ phase (JCPDS No. 28-1192). The absence of secondary phases or impurity peaks confirms the high purity of the product. Notably, the dominance of the β -phase is crucial, since this polymorph is widely recognized to provide much higher upconversion efficiency compared with the cubic α -phase. Stabilization of this phase ensures efficient multiphoton energy transfer when combined with Yb^{3+} sensitizers and $\text{Er}^{3+}/\text{Tm}^{3+}$ activators.

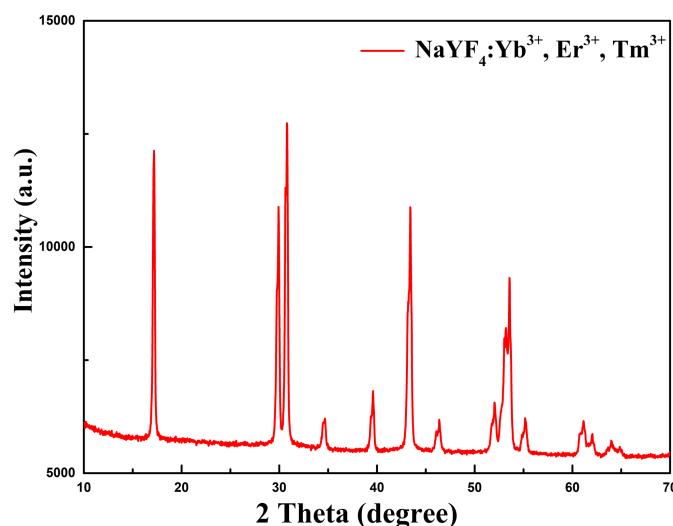


Fig. 2. XRD pattern of $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs showing sharp diffraction peaks corresponding to $\beta\text{-NaYF}_4$ (JCPDS 28-1192).

The single-phase $\beta\text{-NaYF}_4$ diffraction pattern without secondary phases confirms successful isovalent substitution of Y^{3+} by $\text{Yb}^{3+}, \text{Er}^{3+}$, and Tm^{3+} ions.

The morphology of the nanoparticles was examined by FESEM, as shown in Fig. 3. The UCNPs exhibit a nearly spherical shape with narrow size distribution in the range of $\sim 50\text{--}100$ nm. Such particle dimensions are highly favorable for biomedical use, since they facilitate efficient cellular uptake through endocytosis while maintaining good colloidal stability in aqueous environments. The uniform morphology and high crystallinity also help suppress surface-related nonradiative losses, thereby contributing to the pronounced upconversion luminescence observed in subsequent optical studies.

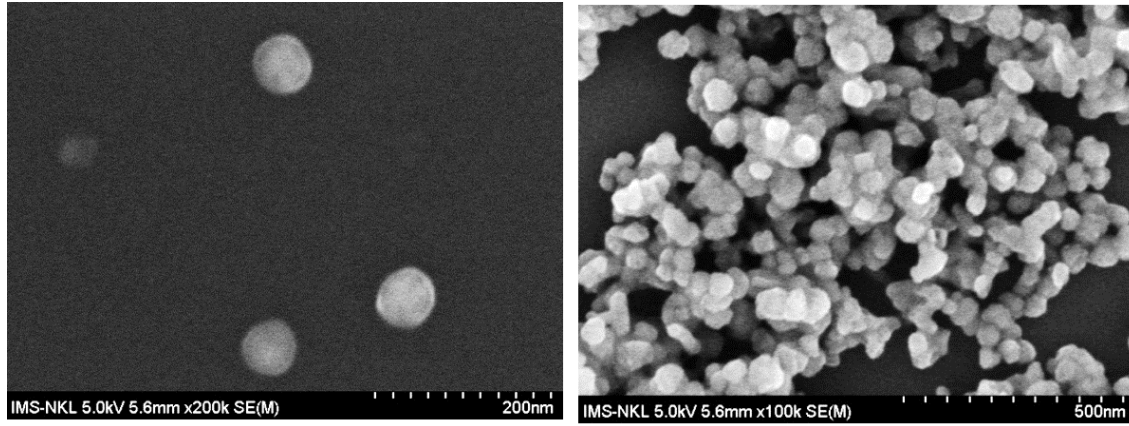


Fig. 3. FESEM images of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs.

3.2. Upconversion luminescence and power-dependent behavior

Upconversion photoluminescence (UCPL) is a nonlinear optical process in which two or more low-energy photons (typically near-infrared, NIR) are absorbed and converted into a single higher-energy photon in the visible region. Power-dependent analysis provides a quantitative probe of this mechanism: by monitoring how emission intensity (I) varies with excitation power (P), one can determine the effective number of photons (n) involved. The relationship generally follows a power-law dependence [26]:

$$I \propto P^n, n = \frac{d(\log I)}{d(\log P)}.$$

Here, n corresponds to the slope of the $\log I$ – $\log P$ plot. Integer values of n indicate the nominal number of photons participating in the process (e.g., $n \approx 2$ for a two-photon process), while deviations from integer values reflect additional effects such as intermediate-state saturation, cross-relaxation, or competing nonradiative decay [30].

Thus, power-dependent UCPL analysis not only quantifies photon participation but also provides insight into energy-transfer dynamics and emission efficiency. The upconversion emission spectra of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs under 980 nm excitation at different powers (300–600 mW) are shown in Fig. 4. Three distinct emission bands are observed: green emission (~ 540 nm) corresponding to the $^2\text{H}_{11/2}$,

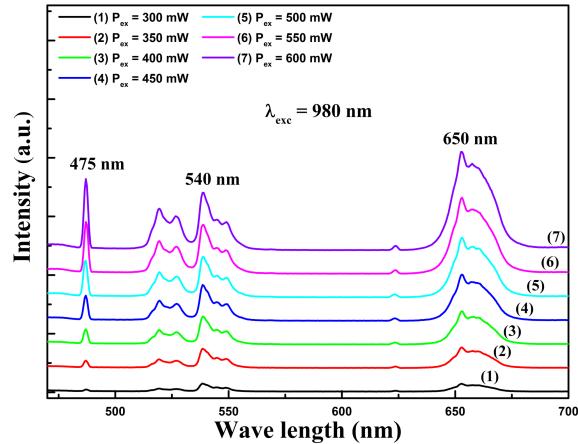


Fig. 4. Upconversion emission spectra of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs under 980 nm excitation at different powers (300–600 mW).

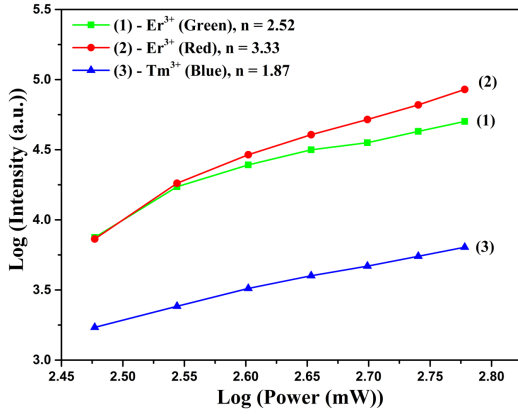


Fig. 5. Log–log plots of upconversion emission intensity versus excitation power for green (~540 nm), red (~650 nm), and blue (~475 nm) channels. Linear fits were used to extract photon orders (n).

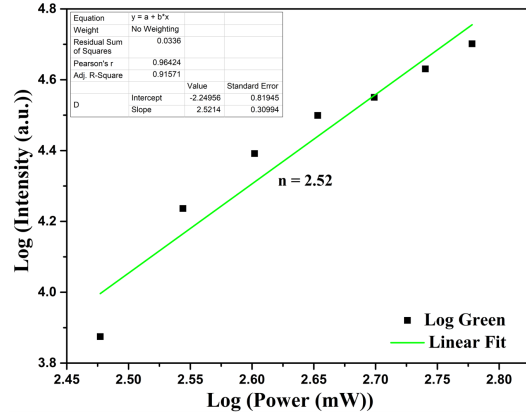


Fig. 6. Log–log plot of green upconversion emission intensity (~540 nm) as a function of excitation power with linear fitting.

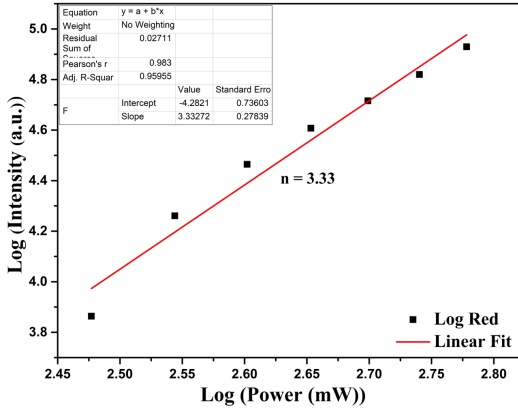


Fig. 7. Log–log plot of red UC emission intensity (~650 nm) as a function of excitation power with linear fitting.

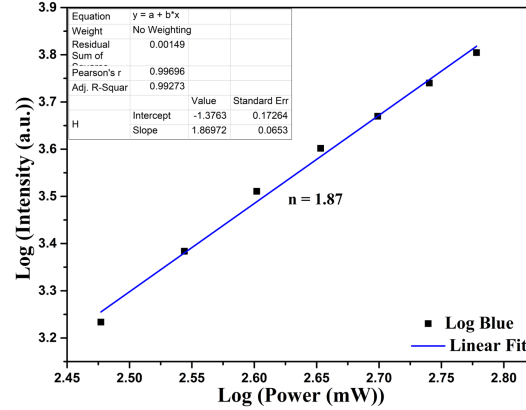


Fig. 8. Log–log plot of blue UC emission intensity (~475 nm) as a function of excitation power with linear fitting.

$^4S_{3/2} \rightarrow ^4I_{15/2}$ transitions of Er^{3+} , red emission (~650 nm) from the $^4F_{9/2} \rightarrow ^4I_{15/2}$ transition of Er^{3+} , and blue emission (~475 nm) from the $^1G_4 \rightarrow 3H_6$ transition of Tm^{3+} . At low excitation power, green and red emissions dominate, while the blue emission becomes more pronounced at higher powers, indicating competitive $\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$ and $\text{Yb}^{3+} \rightarrow \text{Tm}^{3+}$ energy-transfer pathways.

The raw emission intensities of the green (~540 nm), red (~650 nm), and blue (~475 nm) bands, together with their logarithmic values, are summarized in Table 1. These data were used to construct log–log plots (Fig. 5) and to extract the photon orders for each channel.

The log–log plots and linear fits (Figs. 6–8) yielded the following effective photon orders:

- Green (~540 nm): $n \approx 2.52$, confirming a two-photon process.
- Red (~650 nm): $n \approx 3.33$, indicating additional cross-relaxation/ETU contributions.

• Blue (~475 nm): $n \approx 1.87$, close to two-photon behavior in this power range, though typically requiring multiple transfers.

These results confirm that excitation power governs photon participation and channel competition, thereby tuning emission color.

Table 1. Raw emission intensities of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs at different excitation powers (300–600 mW). The table lists the measured intensities of green (~540 nm), red (~650 nm), and blue (~475 nm) bands together with their logarithmic values, which were used for log–log slope analysis.

Power (mW)	log (Power)	Intensity Er^{3+} Green (540 nm)	log (Intensity) Green	Intensity Er^{3+} Red (650 nm)	log (Intensity) Red	Intensity Tm^{3+} Blue (475 nm)	log (Intensity) Blue
300	2.477121	7491	3.874539	7307	3.863739	1712	3.233503
350	2.544068	17234	4.236386	18234	4.260882	2419	3.383636
400	2.602060	24628	4.391429	29125	4.464266	3241	3.510679
450	2.653213	31563	4.499178	40466	4.607090	3996	3.601625
500	2.698970	35498	4.550204	51934	4.715452	4674	3.669689
550	2.740363	42716	4.630591	66011	4.819616	5496	3.740047
600	2.778151	50275	4.701352	85012	4.929480	6376	3.804548

3.3. Intensity ratio analysis

Beyond slope extraction, intensity ratio analysis offers a complementary method to evaluate competition among emission channels and relative population dynamics. Fig. 9 shows the variation of the intensity ratios I_{650}/I_{540} (red-to-green) and I_{475}/I_{540} (blue-to-green) as a function of excitation power.

At low excitation powers, the green emission (~540 nm) dominates, yielding small values of both ratios. As the excitation power increases, the I_{650}/I_{540} ratio increases steadily, reflecting the progressive enhancement of red emission relative to green due to cross-relaxation–assisted ETU among Er^{3+} ions. In contrast, the I_{475}/I_{540} ratio remains very low and nearly constant across the studied power range, indicating the weak contribution of the Tm^{3+} blue channel under these conditions.

Linking Power-Tunable Ratios to Imaging Contrast The extracted photon orders and linear-fit coefficients (R^2) are summarized in Table 2. The green emission exhibits $n \approx 2.52$ ($R^2 = 0.930$), consistent with a two-photon process. The red emission shows $n \approx 3.33$ ($R^2 = 0.966$), indicating the involvement of multi-step ETU and cross-relaxation processes. The blue emission yields $n \approx 1.87$ with the highest linearity ($R^2 = 0.994$), confirming reliable slope extraction even for this weaker channel. Although Tm^{3+} upconversion nominally requires three or more photons, the reduced slope reflects slope compression due to intermediate-state saturation, a phenomenon widely observed in UCPL studies.

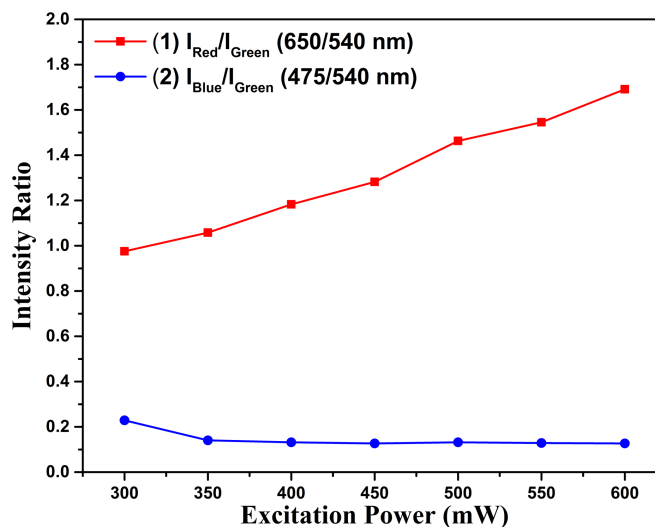


Fig. 9. Intensity ratio plots of I_{650}/I_{540} (red - to - green) and I_{475}/I_{540} (blue-to-green) as a function of excitation power.

Overall, the ratio analysis corroborates the slope results and demonstrates that excitation power serves a dual role: (i) as a mechanistic probe of photon participation and (ii) as a practical control parameter for dynamically tuning emission color. This tunability holds promise for multicolor imaging and ratiometric sensing applications.

Table 2. Extracted photon orders (n) and linear-fit coefficients (R^2) for the three up-conversion emission bands of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Tm}^{3+}$ UCNPs under 980 nm excitation.

Band	Photon order (n)	R^2
Er-green (~ 540 nm)	2.52	0.930
Er-red (~ 650 nm)	3.33	0.966
Tm-blue (~ 475 nm)	1.87	0.994

Moreover, increasing the excitation power selectively enhances the red emission relative to the green band while simultaneously suppressing the blue channel. This behavior originates from distinct population dynamics within the Er^{3+} and Tm^{3+} energy manifolds, where multi-photon Er^{3+} transitions and cross-relaxation processes become increasingly dominant. Such red-weighted spectral redistribution improves imaging contrast in biological environments, as longer wavelengths experience reduced scattering and allow deeper tissue penetration.

3.4. Energy-transfer mechanism

To connect the power-dependent slopes with microscopic processes, the underlying energy-transfer pathways were rationalized using the energy-level diagram of Yb^{3+} , Er^{3+} , and Tm^{3+}

(Fig. 10). Yb^{3+} ions act as sensitizers, absorbing NIR photons and transferring energy to activator ions. The extracted slopes in Table 2 align well with the proposed pathways, providing a coherent picture of photon participation, cross-relaxation, and slope compression.

The extracted photon orders exhibit trends consistent with the excitation pathways illustrated in Fig. 10. For Er^{3+} , sequential $\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$ energy transfer upconversion (ETU) populates the $2\text{H}_{11/2}$ and $4\text{S}_{3/2}$ levels, leading to green emission. The measured slope of $n \approx 2.52$ ($R^2 \approx 0.930$) reflects a near two-photon process with slight saturation of the intermediate state. Red emission (~ 650 nm) originates from population of $4\text{F}_{9/2}$ via multi-step ETU combined with Er^{3+} – Er^{3+} cross-relaxation ($^4\text{I}_{13/2} + ^4\text{I}_{13/2} \rightarrow ^4\text{F}_{9/2} + ^4\text{I}_{15/2}$), consistent with $n \approx 3.33$ ($R^2 \approx 0.966$). For Tm^{3+} , blue emission (~ 475 nm) nominally requires three or more successive $\text{Yb}^{3+} \rightarrow \text{Tm}^{3+}$ transfers; however, the observed $n \approx 1.87$ ($R^2 \approx 0.994$) suggests slope compression due to partial saturation and competition between multiphoton ETU and excited-state absorption (ESA). We emphasize that these mechanistic assignments are consistent with the steady-state data; lifetime-resolved studies will be required for definitive discrimination among ESA, cross-relaxation, and cooperative processes.

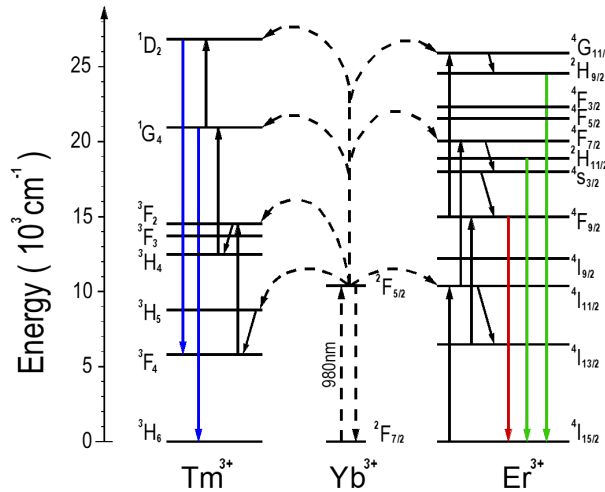


Fig. 10. Energy-level diagram of Yb^{3+} , Er^{3+} , and Tm^{3+} ions under 980 nm excitation, illustrating the pathways responsible for green (~ 540 nm), red (~ 650 nm), and blue (~ 475 nm) upconversion emissions.

In summary, the mechanistic interpretation agrees with the extracted slopes: the green emission is governed by a near two-photon process, the red emission arises from cross-relaxation–assisted ETU with an enhanced photon order, and the blue emission originates from a higher-order Tm^{3+} pathway that shows effective slope compression. This competitive interplay between $\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$ and $\text{Yb}^{3+} \rightarrow \text{Tm}^{3+}$ channels dictates the tunable balance of green, red, and blue emissions, providing a mechanistic basis for exploiting these UCNPs in multicolor imaging, ratio-metric sensing, and theranostic applications.

Although these mechanistic assignments are consistent with well-established lanthanide upconversion behavior and are supported by slope analysis and saturation trends, we acknowledge

that the lack of time-resolved spectroscopic data remains a limitation. Future work will include lifetime-resolved measurements on singly doped and composition-varied samples to further validate the proposed pathways.

3.5. Bioimaging and cytotoxicity in MCF-7 cells

The biomedical potential of the UCNPs was validated in MCF-7 breast cancer cells. As shown in Fig. 11, bright intracellular luminescence confirmed efficient nanoparticle uptake. Signals were localized in the cytoplasm, consistent with endocytosis, while nuclei were counterstained with Hoechst 33342.

The nanoscale size (50–100 nm) and TPGS-modified surface facilitated dispersion and uptake, ensuring uniform intracellular labeling. In the negative control group, no red fluorescence was observed, as expected.

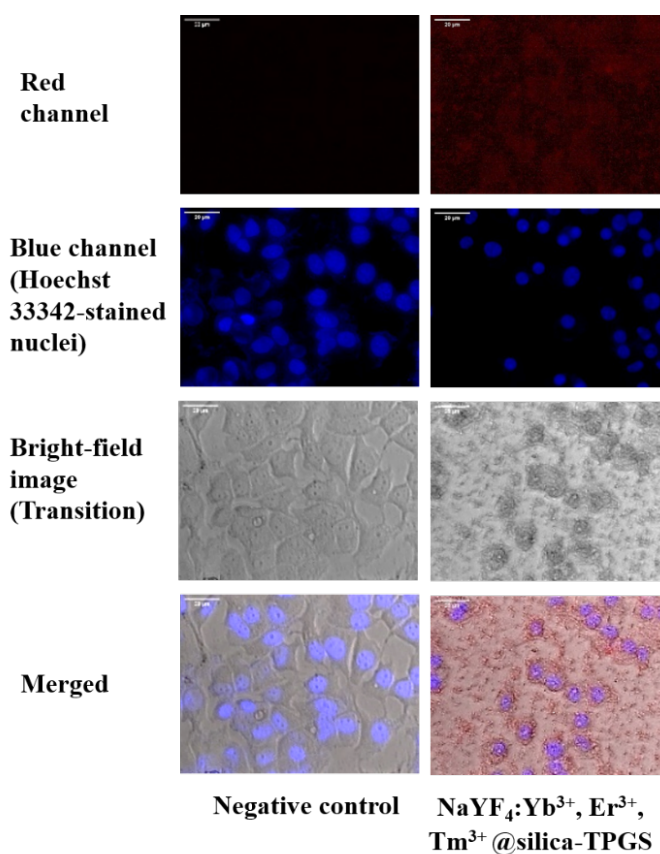


Fig. 11. (Color online) Fluorescence microscopy images of MCF-7 breast cancer cells incubated with negative control (no nanoparticles) and NaYF₄: Yb³⁺, Er³⁺, Tm³⁺ @silica-TPGS biofunctionalized nanoparticles.

Cells treated with $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+},\text{Tm}^{3+}@\text{silica-TPGS}$ nanoparticles exhibited intense red fluorescence localized within the cytoplasm, indicating efficient endocytic uptake and selective intracellular accumulation. The merged fluorescence images revealed clear co-localization of the red (UCNP) and blue (nucleus) signals surrounding the nuclei, with no detectable off-target emission, confirming high labeling specificity.

Compared with organic dyes, the UCNPs exhibited negligible background autofluorescence and outstanding photostability, enabling high-contrast and long-term cellular imaging. Furthermore, their intrinsic capability to modulate the emission profile through excitation power allows multiplexed imaging and dynamic intracellular tracking.

Cytotoxicity was assessed using three independent biological replicates, each performed in triplicate. GI_{50} values were calculated with TableCurve 2D v4 software. A clear dose-dependent inhibition was observed, showing high cell viability ($>90\%$) at low concentrations (≤ 0.032 ppm) and pronounced cytotoxicity at higher doses ($\sim 15\%$ viability at 0.80 ppm). These findings demonstrate that, in addition to imaging capability, the nanoparticles exhibit strong growth-inhibitory activity.

Taken together, these findings highlight the dual functionality of silica-TPGS-coated UCNPs as high-performance luminescent probes and potential anticancer agents, underscoring their strong potential for cancer theranostic applications.

Overall, this study demonstrates that excitation-power-dependent luminescence not only elucidates fundamental energy-transfer mechanisms but also offers a practical approach to optimizing imaging contrast without altering nanoparticle composition. Future work will focus on low-power, real-time live-cell imaging to further validate power-modulated emission under biologically relevant excitation conditions.

4. Conclusion

In conclusion, $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+},\text{Tm}^{3+}@\text{silica-TPGS}$ nanoparticles were successfully synthesized with high crystallinity, uniform morphology, enhanced colloidal stability, and good biocompatibility. Power-dependent luminescence studies clarified photon participation and pathway competition, whereas biological assays confirmed efficient uptake, bright intracellular luminescence, and dose-dependent cytotoxicity with a GI_{50} of 0.26 ± 0.02 ppm against MCF-7 cells. Altogether, these results underscore the potential of excitation-power-controlled UCNPs as multifunctional platforms for ratiometric sensing, multicolor imaging, and cancer theranostics.

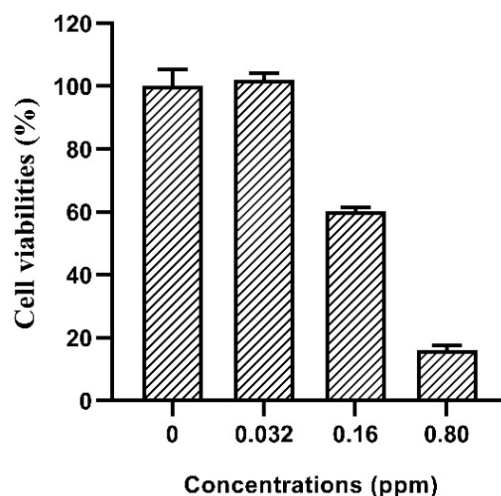


Fig. 12. Cytotoxicity results (SRB assay) of $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+},\text{Tm}^{3+}@\text{silica-TPGS}$ biofunctionalized nanoparticles against MCF-7 cells - GI_{50} : 0.26 ± 0.02 ppm.

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Authors contributions

Tran Thu Huong: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review and editing, and preparation of the final manuscript. Ha Thi Phuong: Methodology, Investigation, Formal analysis, Data curation. Le Thi Vinh: Conceptualization, Methodology, Investigation, Formal analysis. Hoang Thi Khuyen: Methodology, Investigation. Pham Thi Lien: Methodology, Formal analysis. Nguyen Thi Nga: Investigation, Formal analysis, Data curation. Do Thi Phuong: Investigation, Formal analysis, Data curation. Do Thi Thao: Methodology, Investigation, Formal analysis.

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there is no conflicts of interest regarding the publication of this paper.

References

- [1] M. Haase and H. Schäfer, *Upconverting Nanoparticles*, *Angew. Chem. Int. Ed.* **50** (2011) 5808.
- [2] G. Chen, H. Qiu, P. N. Prasad and X. Chen, *Upconversion Nanoparticles: Design, Nanochemistry, and Applications*, *Chem. Rev.* **114** (2014) 5161.
- [3] X. Wu, G. Chen, J. Shen, Z. Li, Y. Zhang and G. Han, *Upconversion Nanoparticles: A Versatile Solution to Multiscale Biological Imaging*, *Bioconjugate Chem.* **26** (2015) 166.
- [4] H. S. Qian and Y. Zhang, *Synthesis of hexagonal-phase core-shell NaYF₄ nanocrystals with tunable upconversion fluorescence*, *Langmuir* **24** (2008) 12123.
- [5] J. Zhou, Z. Liu and F. Li, *Upconversion nanophosphors for small-animal imaging*, *Chem. Soc. Rev.* **41** (2012) 1323.
- [6] F. Wang and X. Liu, *Upconversion Multicolor Fine-Tuning: Visible to Near-Infrared Emission from Lanthanide-Doped NaYF₄ Nanoparticles*, *J. Am. Chem. Soc.* **130** (2008) 5642.
- [7] C. M. S. Jones, A. Gakamsky and J. M. Hueso, *The upconversion quantum yield (UCQY): a review to standardize the measurement methodology, improve comparability, and define efficiency standards*, *Sci. Technol. Adv. Mater.* **22** (2021) 811.
- [8] M. V. DaCosta, S. Doughan, Y. Han and U. J. Krull, *Lanthanide upconversion nanoparticles and applications in bioanalysis*, *Anal. Chim. Acta* **832** (2014) 1.
- [9] Z. Gerelkhuu, Y. Lee and T. H. Yoon, *Upconversion Nanomaterials in Bioimaging and Biosensor Applications and their biological response*, *Nanomaterials* **12** (2022) 3470.
- [10] M. C. Cinar, M. S. Gocmen, A. Aciksari, R. Ceylan, S. Sahsuvar, S. Cetinel *et al.*, *UCNP-based targeted imaging of MCF-7 cells*, *J. Nanoparticle Res.* **26** (2024) 122.
- [11] L. Gaofeng, W. Haojie, S. Hao, W. Haitao, Z. Mengxi, J. Aihua *et al.*, *Recent progress in the development of upconversion nanomaterials in bioimaging and disease treatment*, *J. Nanobiotechnol.* **18** (2020) 154.
- [12] J. Zhou, Q. Liu, W. Feng, Y. Sun and F. Li, *Upconversion Luminescent Materials: Advances and Applications*, *Chem. Rev.* **115** (2015) 395.
- [13] Y. Liu, D. Tu, H. Zhu, E. Ma and X. Chen, *Lanthanide-doped luminescent nano-bioprobes: from fundamentals to biodetection*, *Nanoscale* **5** (2013) 1369.
- [14] T. K. V. Le, B. T. Nguyen, T. M. D. Cao, T. T. V. Tran *et al.*, *Up conversion luminescence in Er and Tm activated NaYF₄ microparticles*, *Optical Materials* **149** (2024) 115099.

- [15] D. Pominova, V. Proydakova, I. Romanishkin, S. Kuznetsov, K. Linkov, N. Tabachkova *et al.*, *NaGdF₄:Yb,Er,Tm UCNPs for bioimaging in shortwave-infrared range*, *Photonics* **11** (2024) 38.
- [16] C. Würth, B. Grauel, M. Pons, F. Frenzel, P. Rissiek, K. Rücker *et al.*, *Yb- and Er concentration dependence of the upconversion luminescence*, *Nano Res.* **15** (2022) 9639.
- [17] M. Wang, G. Abbineni, A. Clevenger, C. Mao and S. Xu, *Upconversion Nanoparticles: Synthesis, Surface Modification and Biological Applications*, *Nanomedicine* **7** (2012) 710.
- [18] Y. Fan, L. Liu and F. Zhang, *Exploiting lanthanide-doped upconversion nanoparticles with core/shell structures*, *Nano Today* **25** (2019) 68.
- [19] H. Li, H. Liu, K. L. Wong and A. H. All, *Lanthanide-doped upconversion nanoparticles as nanoprobes for bioimaging*, *Biomater. Sci.* **12** (2024) 4650.
- [20] E. Voronovic, A. Skripka, G. Jarockyte, M. Ger, D. Kuciauskas, A. Kaupinis *et al.*, *Uptake of Upconverting Nanoparticles by Breast Cancer Cells*, *ACS Appl. Mater. Interfaces* **13** (2021) 39076.
- [21] A. Satpathy, T. J. Su, W. T. Huang, C. J. Chiang and R. S. Liu, *Versatile Nanoplatforms for Bioimaging and Therapy Using UCNPs*, *ACS Appl. Opt. Mater.* **2** (2024) 1790.
- [22] X. D. Wang, Z. X. Shen, T. Sang, X. B. Chen, M. F. Li, L. Y. Chen and Z. S. Wang, *Preparation of spherical silica particles by Stöber process*, *J. Colloid Interface Sci.* **341** (2010) 23.
- [23] H. T. Phuong, L. T. Vinh, T. Q. Cong, T. Q. Tien, N. D. Van, V. T. H. Ha *et al.*, *Optimized NaYF₄:Er³⁺/Yb³⁺ Upconversion Nanocomplexes via Oleic Acid for Biomedical Applications*, *Inorganics* **13** (2025) 140.
- [24] S. C. Brooks, E. R. Locke and H. D. Soule, *Estrogen reception in a human cell line (MCF-7) from a breast carcinoma*, *J. Biol. Chem.* **248** (1973) 6251.
- [25] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica *et al.*, *New colorimetric cytotoxicity assay for anticancer drug screening*, *J. Natl. Cancer Inst.* **82** (1990) 1107.
- [26] M. Pollnau, D. R. Gamelin, S. R. Lüthi, H. U. Güdel and M. P. Hehlen, *Power dependence of upconversion luminescence*, *Phys. Rev. B* **61** (2000) 3337.
- [27] K. Zheng, L. Wang, D. Zhang, D. Zhao and W. Qin, *Power switched multiphoton upconversion emissions*, *Opt. Express* **18** (2010) 2934.
- [28] A. Shyichuk, S. S. Câmara, I. T. Weber and A. C. Neto, *Energy-transfer UC dynamics YVO₄:Yb³⁺,Er³⁺*, *J. Luminescence* **170** (2025) 560.
- [29] J. F. Suyver, A. Aebischer, S. García-Revilla, P. Gerner and H. U. Güdel, *Novel materials doped with lanthanides beyond 4f-4f transitions*, *Phys. Rev. B* **71** (2005) 125123.
- [30] J. C. Boyer and C. J. M. V. Frank, *Absolute quantum yield measurements of colloidal NaYF₄:Er³⁺,Yb³⁺*, *Nanoscale* **2** (2010) 1417.
- [31] T. T. Huong, H. T. Phuong, L. Th. Vinh, H. T. Khuyen, D. T. Thao, L. D. Tuyen *et al.*, *Upconversion NaYF₄:Yb³⁺/Er³⁺@silica bio-nano complexes*, *J. Phys. Chem. B* **125** (2021) 9768.
- [32] H. T. Phuong, T. T. Huong, L. T. Vinh, D. T. Thao, L. A. Tu, T. Q. Cong *et al.*, *SCN-IgG functionalized NaYF₄:Yb³⁺/(Er³⁺,Tm³⁺) upconversion nanoparticles*, *RSC Adv.* **15** (2025) 22682.
- [33] K. Cheng, Q. Luan, T. Liu, B. Albinsson and L. Hou, *Semiconductor nanocrystals-based photon-upconversion*, *Responsive Mater.* **3** (2025) e20240030.
- [34] A. Nsubuga, N. Fayad, F. Pini and M. M. Natile, *Small upconversion-ruthenium nanohybrids for cancer therapy*, *Nanoscale* **17** (2025) 3809.
- [35] C. Kembuan, H. Oliveira and C. Graf, *Effect of different silica coatings on the toxicity of upconversion nanoparticles*, *Beilstein J. Nanotechnol.* **12** (2021) 35.