

Supplementary materials

For

Assessing the effects of luminescently labelled and non-labelled PET nanoparticles on environmental bacteria

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1. Synthesis of UCNPs

The core@shell upconverting nanoparticles (UCNPs) were obtained according to the modified procedure described by Homann et al (Homann et al., 2018). The core@shell β -NaYF₄:18%Yb³⁺,2%Er³⁺@ β -NaYF₄ UCNPs were prepared from rare earths acetates (ytterbium and erbium acetates obtained in our laboratory) in the fourth steps:

- I Synthesis of α -NaYF₄:18%Yb³⁺,2%Er³⁺ core
- II Synthesis of β -NaYF₄:18%Yb³⁺,2%Er³⁺ core from synthesised α -particles in the first step
- III Synthesis of α -NaYF₄ shell, analogous to the first step,
- IV Synthesis of β -NaYF₄:18%Yb³⁺,2%Er³⁺@ β -NaYF₄ core@shell UCNPs, due to mixing β -core (second step) and α -shell (third step)

1.1 Synthesis of anhydrous rare earths acetates

The anhydrous acetates of ytterbium and erbium were synthesised starting from their respective rare earth oxides. A total of 10 mmol of ytterbium oxide was mixed with 40 mL of acetic acid and 40 mL of distilled water in a 250 mL three-neck round-bottom flask. The mixture was heated at 90 °C under a reflux condenser for approximately 12 hours until the oxide fully dissolved and a clear solution was obtained. After cooling to room temperature, the solvent was nearly completely removed using a rotary evaporator, resulting in an almost dry powder. Therefore, approximately 30 mL of acetic acid was added to the powder, and the mixture was stirred and gently heated to around 60 °C using the rotary evaporator. The resulting solution of hydrated ytterbium acetates was transferred to a three-neck round-bottom flask equipped with a dropping funnel and reflux condenser. The solution was heated to 120 °C, after which 10 mL of anhydrous acetic acid was slowly added via the dropping funnel. The reaction mixture was maintained at a temperature above the boiling point of glacial acetic acid (approx. 120 °C) for 2 hours. Following this, the mixture was cooled to room temperature, and the solvent was

removed by rotary evaporation to obtain a dry powder. Thermogravimetric analysis (TGA) was performed on the final product to assess the residual water content. The synthesis of anhydrous erbium acetate was carried out using the same procedure.

1.2 Synthesis of α -NaYF₄:18%Yb³⁺,2%Er³⁺ core

The mixture of oleic acid and 1-octadecene (10 mL of each solvent per 1 mmol of product) was placed in a three-neck round-bottom flask equipped with a thermosensor and connected to a Schlenk line.

The solution was degassed under vacuum at room temperature and heated up to 100 °C for 60 minutes under reduced pressure (<1 mbar). After cooling to room temperature, rare earth acetates – yttrium, ytterbium, and erbium were added in a molar ratio of 0.8:0.18:0.02. The resulting mixture was again degassed under vacuum at room temperature and heated to 100 °C for another 60 minutes (<1 mbar). While maintaining an N₂ atmosphere, sodium oleate was introduced into the hot solution in a 2.8:1 molar ratio relative to total rare earth ions and degassed to complete dissolution (20 min). Next, ammonium fluoride was added in a 5.8:1 molar ratio per 1 mmol of rare earth ions. The reaction setup was then cycled three times between vacuum and nitrogen for 5 seconds on each cycle to ensure the complete removal of air and moisture. Subsequently, the mixture was heated to 200 °C under nitrogen and maintained at this temperature for 60 minutes. After cooling to room temperature, the resulting particles were isolated by centrifugation, and sodium fluoride by-products were removed. The particles were further purified by precipitation with ethanol, separation via centrifugation, and washing with hexane. The final product was dried at room temperature and used promptly.

1.3 Synthesis of β -NaYF₄:18%Yb³⁺,2%Er³⁺ core

The α -NaYF₄:18%Yb³⁺, 2%Er³⁺ core precursor was mixed with oleic acid and 1-octadecene (3.5 mL of each solvent per 1 mmol of core precursor), placed in a three-neck round-bottom flask equipped with a thermosensor and connected to a Schlenk line. The mixture was degassed under vacuum at room temperature and then heated to 100 °C for 90 minutes under reduced pressure (<1 mbar). Following degassing, the reaction mixture was heated to 300 °C and maintained at this temperature for 80 minutes before being allowed to cool to room temperature. The resulting solution was mixed with hexane and ethanol in a 1:1:1 volume ratio and centrifuged to isolate the precipitate. The solid was redispersed in hexane, and an equal volume of ethanol was added to induce reprecipitation. The nanoparticles were finally collected by centrifugation and dried at room temperature.

1.4 Synthesis of α -NaYF₄ shell

Synthesis of α -NaYF₄ shell was conducted in the same way as α -NaYF₄:18%Yb³⁺, 2%Er³⁺ core, with some changes; only yttrium acetate was added to the degassed mixture of oleic acid and 1-octadecene, sodium oleate and ammonium fluoride were added in the stoichiometric ratio.

1.5 Synthesis of β -NaYF₄:18%Yb³⁺,2%Er³⁺@ β -NaYF₄ core@shell

The mixture of oleic acid and 1-octadecene containing core particles and shell precursor (core to shell ratio 1:7, 2.5 mL of each solvent per 1 mmol of product) was placed in a three-neck round-bottom flask and connected to a Schlenk line. The solution was degassed by stirring under vacuum (<1 mbar) at 100 °C for 120 minutes. Following degassing, the mixture was

heated to 300 °C and maintained at that temperature for 140 minutes, then allowed to cool to room temperature. The resulting solution was mixed with hexane and ethanol in a 1:1:1 volume ratio and centrifuged to isolate the solid phase. The precipitate was redispersed in hexane, followed by adding an equal volume of ethanol to induce reprecipitation and subsequent centrifugation. Finally, the nanoparticles were dispersed in hexane and stored at 5–7 °C.

2. Synthesis of Eu(TTA)₃

The complex of europium thenoyltrifluoroacetone, as the dihydrate [Eu(TTA)₃·2H₂O], was prepared according to the literature procedure (Binnemans et al., 2004). Initially, 2-thenoyltrifluoroacetone (0,67 g; 3 mmol) was dissolved in ethanol (15 mL). Then, the 0.1 M solution of EuCl₃ (10 mL) and 1M NaOH (3 mL) was added dropwise into the reaction flask. Afterwards, 100 mL of redistilled water was also added to the other reactants. The final reaction mixture was stirred for several minutes at 60°C. The pale yellow powder started to precipitate during the cooling reaction mixture to room temperature. The obtained product was filtered off, washed with a small amount of redistilled water and dried at 50°C for 24h.

3. Characteristics of UCNPs

DLS (Dynamic Light Scattering) analysis was carried out using Zetasizer Nano (Malvern Instruments Ltd, Malvern, UK).

HR-TEM (High-resolution Transmission Electron Microscopy) imaging was performed using an FEI (S)TEM Titan G2 600–300 Hitachi HT7700 microscope to check the size and morphology of the UCNPs obtained.

X-ray diffraction (XRD) spectra were taken using a Bruker AXS D8 Advance X-ray diffractometer with Cu K α radiation ($\lambda = 0.15406$ nm) (Bruker Corporation, Billerica, USA) to confirm the chemical composition of UCNPs obtained.

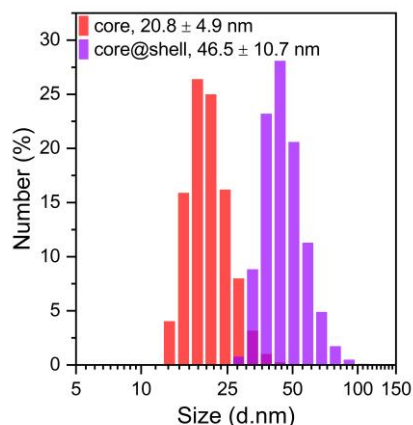


Figure S1. Diameter measurement of UCNPs by DLS technique.

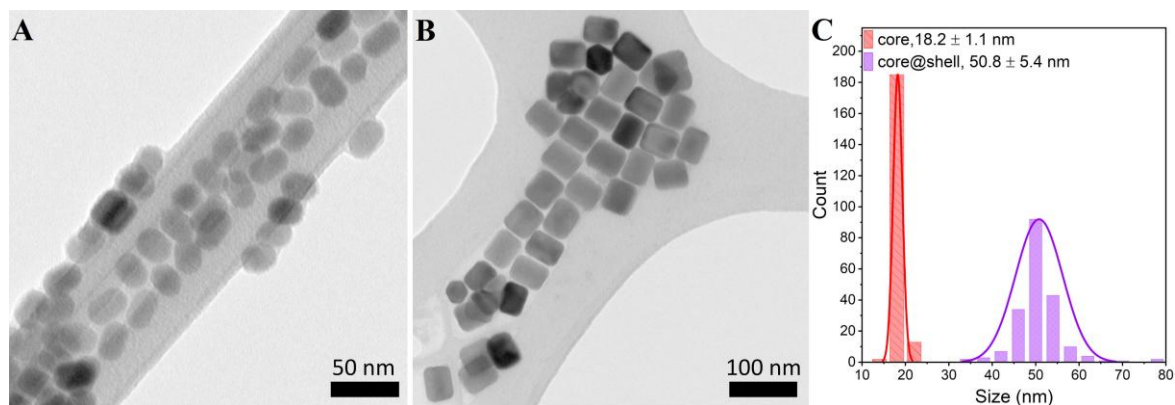


Figure S2. TEM images (A core, B core@shell) and sizes of UCNPs (C) determined from TEM images.

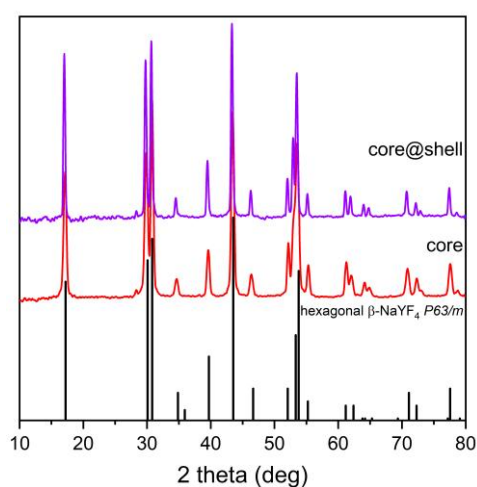


Figure S3. XRD spectra of UCNPs together with the reference pattern of hexagonal β -NaYF₄ (no. 51916) reported in the Inorganic Crystal Structure Database (ICSD).

4. XRD of nanoPET and nanoPET+UCNPs

The resulting PET materials (nanoPET and nanoPET+UCNPs) were also subjected to XRD analysis.

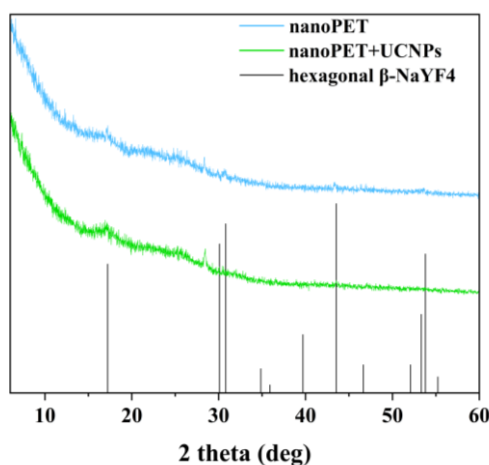


Figure S4. XRD spectra of nanoPET and nanoPET+UCNPs together with the reference pattern of hexagonal β -NaYF₄ (no. 51916) reported in the Inorganic Crystal Structure Database (ICSD).

5. FTIR of nanoPETs

IR spectra were taken using the Vertex 70 (Bruker, Germany) Spectrometer equipped with a MIRacle™ ATR accessory (single reflection diamond ATR crystal). The analysis helped to establish the absence of hexafluoroisopropanol and the success of the purification method.

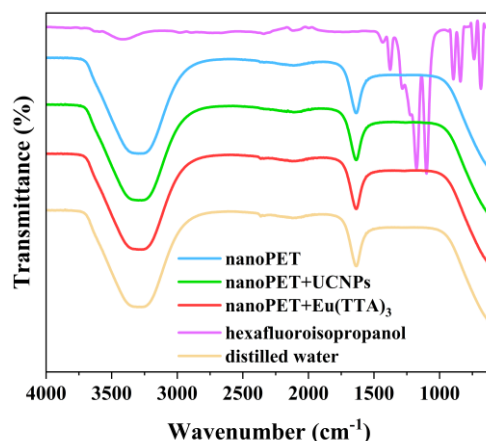


Figure S5. FTIR spectra of purified nanoPET, nanoPET+UCNPs, nanoPET+Eu(TTA)₃ colloids, pure hexafluoroisopropanol and distilled water.

6. DLS of nanoPETs

The hydrodynamic particle diameter distribution of the prepared nanoPETs was determined using DLS analysis.

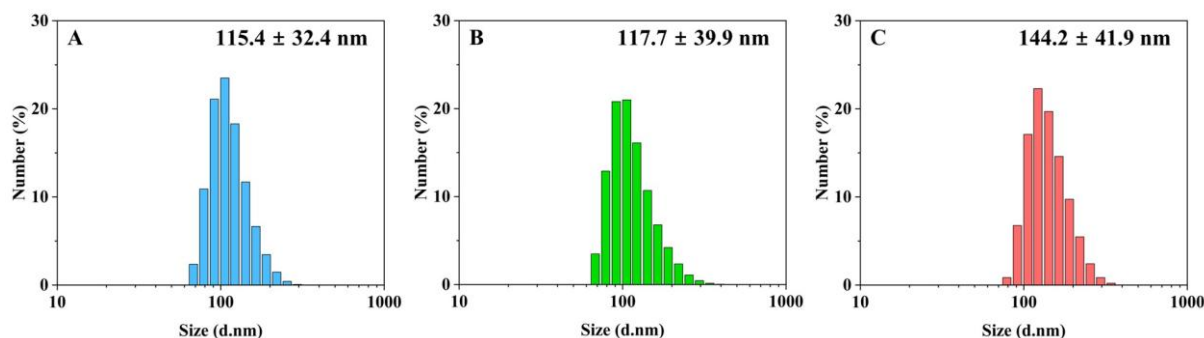


Figure S6. DLS measurement of the hydrodynamic diameter of nanoPETs in prepared colloids: nanoPET (A), nanoPET+UCNPs (B) and nanoPET+Eu(TTA)₃ (C).

7. References

- Binnemans, K., Lenaerts, P., Driesen, K., Görrler-Walrand, C., 2004. A luminescent tris(2-thenoyltrifluoroacetato)europium(III) complex covalently linked to a 1,10-phenanthroline-functionalised sol-gel glass. *J Mater Chem* 14, 191–195. <https://doi.org/10.1039/B311128H>
- Homann, C., Krukewitt, L., Frenzel, F., Grauel, B., Würth, C., Resch-Genger, U., Haase, M., 2018. NaYF₄:Yb,Er/NaYF₄ Core/Shell Nanocrystals with High Upconversion Luminescence Quantum Yield. *Angew. Chem. Int. Ed.* 57, 8765–8769. <https://doi.org/10.1002/anie.201803083>