

Supporting Information

Pr³⁺ doped NaYF₄ and LiYF₄ nanocrystals combining Visible-to-UVC upconversion and NIR-to-NIR-II downconversion luminescence emissions for biomedical applications

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SI 1 Materials and Instruments

Materials

Y₂O₃ (99.99%), Yb₂O₃ (99.99%), Pr₂O₃ (99.99%), LiCO₃ (≥ 90.0%), NaOH (≥ 98.0%), NH₄F (≥ 99.99%), acetic acid (CH₃COOH) (99.5% - 99.9% Pure P.A.), trifluoroacetic acid (CF₃COOH) (reagent grade, ≥ 99%), oleic acid (technical grade, > 93%), 1-octadecene, ethanol, chloroform, acetone and methanol used for NaYF₄ and LiYF₄ syntheses were purchased from Sigma Aldrich and used without further purification. Salmon sperm dsDNA was purchased from Sigma Aldrich and used without further purification.

Instruments

Powder X-ray diffraction (PXRD) patterns were measured with an STOE X-ray diffractometer with Ge-filtered CuK_{α1} radiation. The measurements were done in the Bragg-Brentano geometry in the 10-120° 2θ range.

Transmission electron microscopy (TEM) images were recorded using an FEI Tecnai G² 20 X-TWIN transmission electron microscope. Diluted samples were drop-cast on the dedicated Cu grids covered with carbon and left for solvent evaporation.

Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) Agilent 5110 was used to determine the lanthanide content (operation parameters: RF power – 1.50 kW; Plasma Ar flow rate - 12.00 L/min; Nebulizing Ar flow rate - 0.70 L/min; Auxiliary Ar flow rate - 1.00 L/min; Uptake delay time – 10 s; Read time – 5 s; Number of replicates – 3; Stabilization time – 15 s; Viewing mode – SVDS; Viewing height – 8 mm; Pump speed – 12 rpm; background correction – off-peak, fitted.)

The UVC emission spectra were measured with excitation by a continuous wave 447 nm fiber-coupled semiconductor diode laser (CNI MDL-XD-447), focused with a 200 mm focal length lens (power density at the focal point ~2000 W/cm²). The excited emission was collected with a couple of UV-grade fused silica lenses. A set of two short pass filters with cut-off wavelengths at 400 and 425 nm (Edmund Optics #64-661 and #64-608, respectively) along with a UV bandpass filter (Thorlabs FGUV5-UV) were put in the optical axis to prevent scattered laser

light entering the detection setup. Emission spectra were analyzed with a 320 mm focal length grating (1800 gr./mm, blaze wavelength 330 nm) monochromator (Horiba iHR320) coupled with a pre-amplified photomultiplier tube (Hamamatsu H5784-04). Synchronous phase-sensitive detection was employed to improve the sensitivity of the setup by modulating the laser light with a mechanical chopper at 40 Hz, allowing measurement of emission intensity, represented by the photomultiplier module output voltage, with a lock-in amplifier (EG&G 7260). The experiments were performed at ambient conditions using corresponding nanocrystal powders deposited on glass slides.

Near-infrared emission spectra were acquired with a fiber-coupled, thermoelectrically cooled InGaAs CCD spectrometer (Hamamatsu C9913GC), with a two-lens optical system used for collecting the photoluminescence and coupling it to the fiber. A 1050 nm long-pass filter was used to cut off the scattered laser light (447/980 nm). The emission spectra are recorded by dispersing samples in chloroform solvent (ca. 100mg/mL).

The UV-vis absorption spectra were measured using a JASCO V650 spectrometer with a scan rate of 400 nm/min.

The fluorescence spectra were measured using F-4500 Fluorescence Spectrophotometer with a scan rate 100 nm/min.

SI 2 Syntheses and Characterizations

Syntheses

NaYF₄ NP series: The hexagonal structure β -*NaYF₄* NCs co-doped with Pr³⁺ and Yb³⁺ ions were synthesized based on a protocol described previously.^{1,2} First, the lanthanide acetate precursor was prepared starting from lanthanide oxides (2 mmol of Ln₂O₃ (Ln = Y, Pr, Yb)) and acetic acid, with the use of a microwave reactor (MAGNUM II ERTEC). The obtained dried acetate precursor salt was mixed under an inert atmosphere at 140 °C with 12 mL of oleic acid and 30 mL of 1-octadecene to form a clear, homogenous solution. After initial degassing, the reaction temperature was lowered to 50 °C and methanol solution of ammonium fluoride (8 mmol) and sodium hydroxide (5 mmol) were added dropwise. The obtained mixture was maintained at 60 °C for 45 min followed by the methanol evaporation. Next, the reaction temperature was increased to 300 °C for the synthesis of co-doped β -*NaYF₄* NCs for 60 min under an inert atmosphere. After the NCs formation, the mixture was allowed to cool down to room temperature, followed by the precipitation with ethanol and a small amount of acetone, and isolated by centrifugation. After washing three times with excess amount of ethanol, the obtained NCs were dispersed in 5 mL of chloroform.

Table S1: Summary for the amount of Y₂O₃, Yb₂O₃ and Pr₂O₃ used in the reactions

1 mol%Pr, x mol%Yb x =	Y ₂ O ₃	Yb ₂ O ₃	Pr ₂ O ₃
5	1.88 mmol	0.1 mmol	0.02 mmol
10	1.78 mmol	0.2 mmol	0.02 mmol
20	1.58 mmol	0.4 mmol	0.02 mmol
40	1.18 mmol	0.8 mmol	0.02 mmol
60	0.78 mmol	1.2 mmol	0.02 mmol

LiYF₄ NP series: The hexagonal structure β -*LiYF₄* NCs co-doped with Pr³⁺ and Yb³⁺ ions were synthesized based on a reported protocol.³ The lanthanide trifluoro-acetate precursor was prepared starting from the conversion of lanthanide oxides (1.25 mmol of Ln₂O₃ (Ln = Y, Pr, Yb)), lithium carbonate (2.25 mmol of Li₂CO₃), and an excess of trifluoroacetic acid. The obtained dried trifluoroacetate precursor salt was mixed under an inert atmosphere at 140 °C with 30 mL of oleic acid and 30 mL of 1-octadecene to form a clear, homogenous solution. The

mixture solution was then dried under vacuum for about 30 min to remove O₂ and H₂O. The reactant was heated up to 320 °C slowly (c.a. 5 °C/min rate) and reacted for 1 h under N₂. The resulting solution was then cooled down to room temperature and quenched with ethanol and a small amount of acetone to obtain the corresponding LiYF₄:Pr³⁺/Yb³⁺ NCs and isolated via centrifugation. After washing three times with EtOH, the obtained NCs were dispersed in 5 mL of chloroform.

Table S2: Summary for the amount of Y₂O₃, Yb₂O₃ and Pr₂O₃ used in the reactions

2 mol%Pr, x mol%Yb x =	Y ₂ O ₃	Yb ₂ O ₃	Pr ₂ O ₃
2	1.200 mmol	0.025 mmol	0.025 mmol
5	1.161 mmol	0.063 mmol	0.025 mmol
10	1.101 mmol	0.125 mmol	0.025 mmol
20	0.975 mmol	0.250 mmol	0.025 mmol

Characterizations

All TEM images of $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs showed hexagonal-like shapes with average sizes ca. 80 nm and $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs presented tetrahedral shapes with average sizes ca. 90 nm.

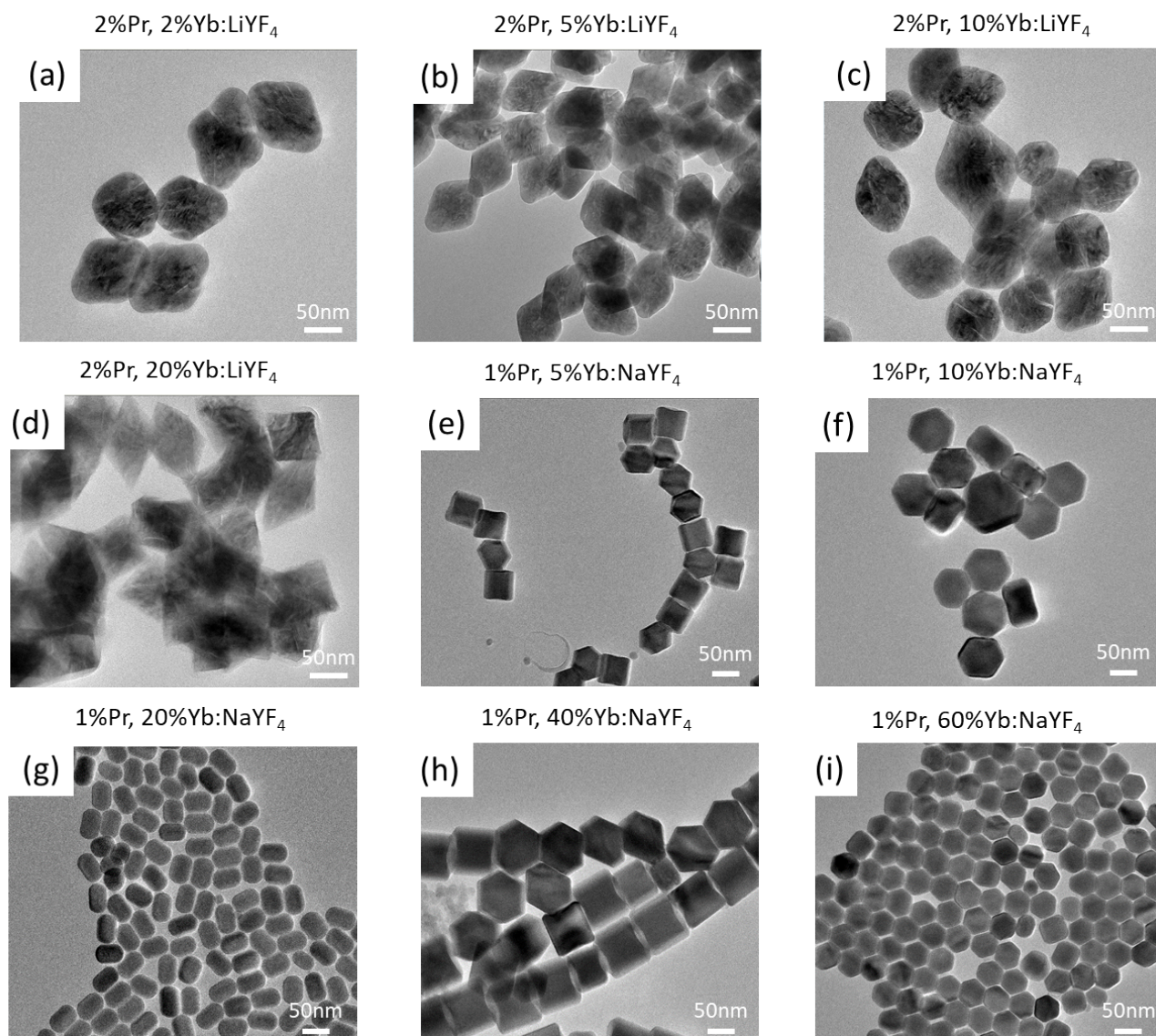


Figure S1: TEM images of (a-d) $\text{LiYF}_4:2 \text{ mol}\% \text{Pr}^{3+}/x \text{ mol}\% \text{Yb}^{3+}$ ($x=2, 5, 10, 20$) and (e-f) $\text{NaYF}_4:1 \text{ mol}\% \text{Pr}^{3+}/x \text{ mol}\% \text{Yb}^{3+}$ ($x = 5, 10$).

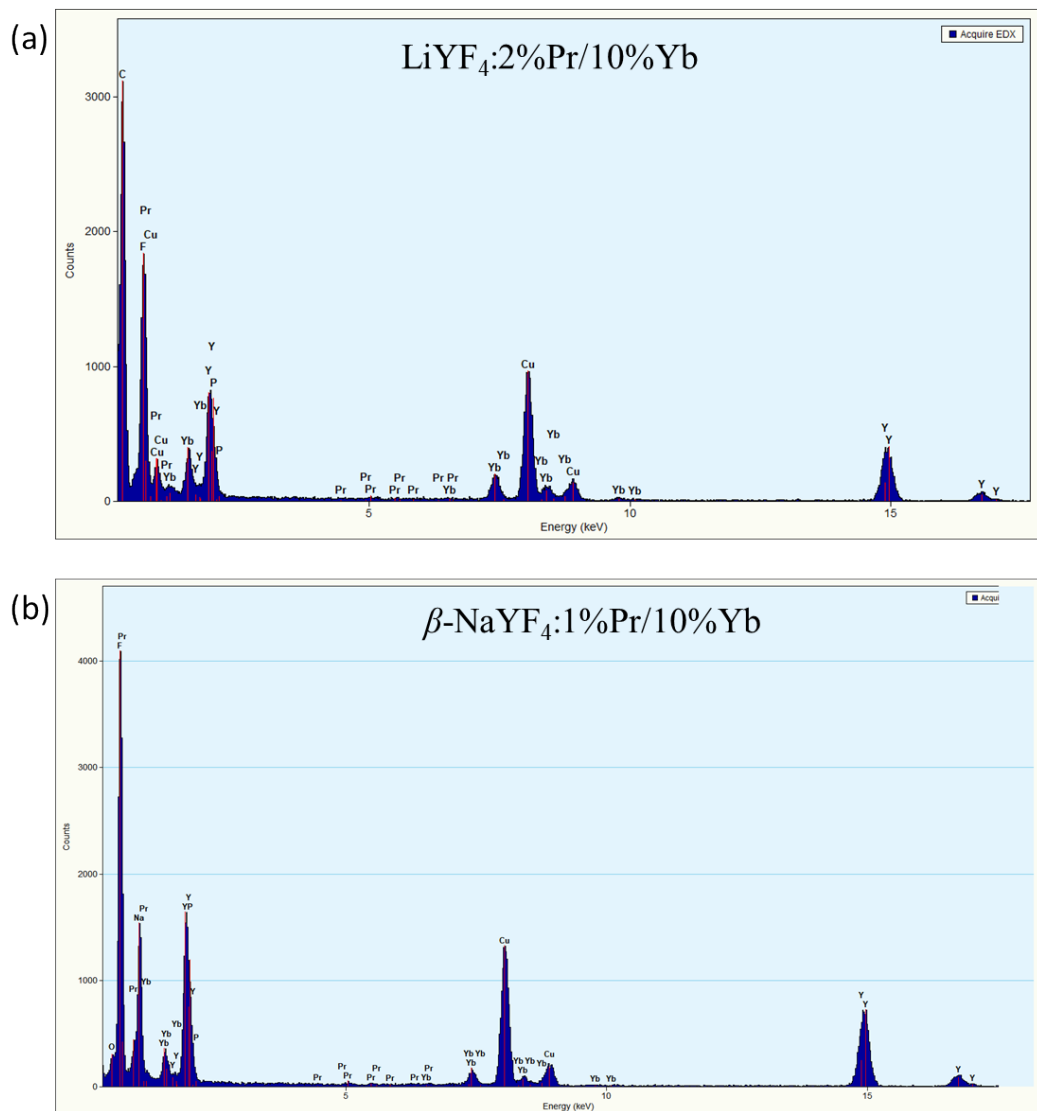


Figure S2: Representative EDX spectra of a) $\text{LiYF}_4:2 \text{ mol}\% \text{Pr}^{3+}/10 \text{ mol}\% \text{Yb}^{3+}$ and b) $\text{NaYF}_4:1 \text{ mol}\% \text{Pr}^{3+}/10 \text{ mol}\% \text{Yb}^{3+}$.

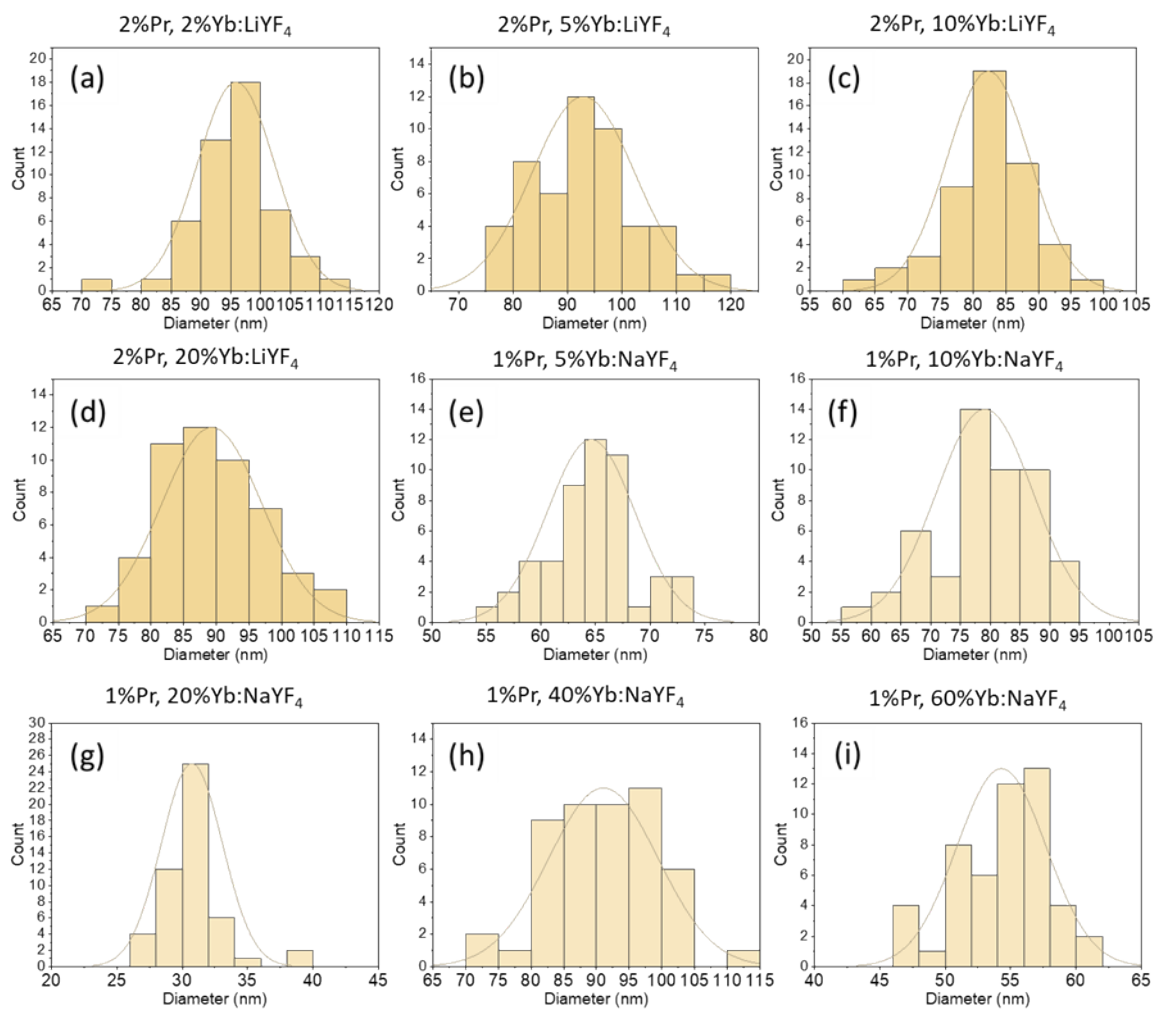


Figure S3: Size distribution histograms from TEM images of the different series of LiYF_4 :

$\text{Pr}^{3+}/\text{Yb}^{3+}$ and NaYF_4 : $\text{Pr}^{3+}/\text{Yb}^{3+}$.

All PXRD patterns of β -NaYF₄:Pr³⁺/Yb³⁺ and LiYF₄:Pr³⁺/Yb³⁺ could be ascribed to the formation of the corresponding hexagonal β -NaYF₄ and tetrahedral LiYF₄ (**Figure S1**).

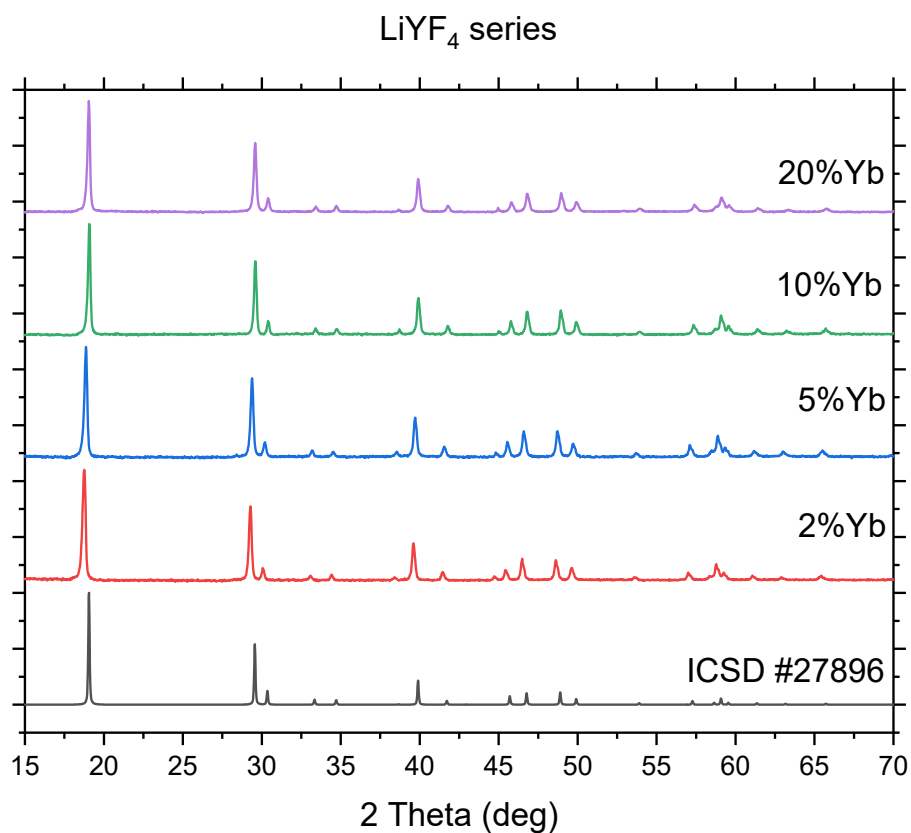


Figure S4: PXRD pattern of LiYF₄:Pr³⁺/Yb³⁺ series with fixed amount of Pr³⁺ and different Yb³⁺ concentration (2, 5, 10, 20 mol%). All PXRD patterns could be ascribed to the formation of the LiYF₄ host (ICSD # 27896).

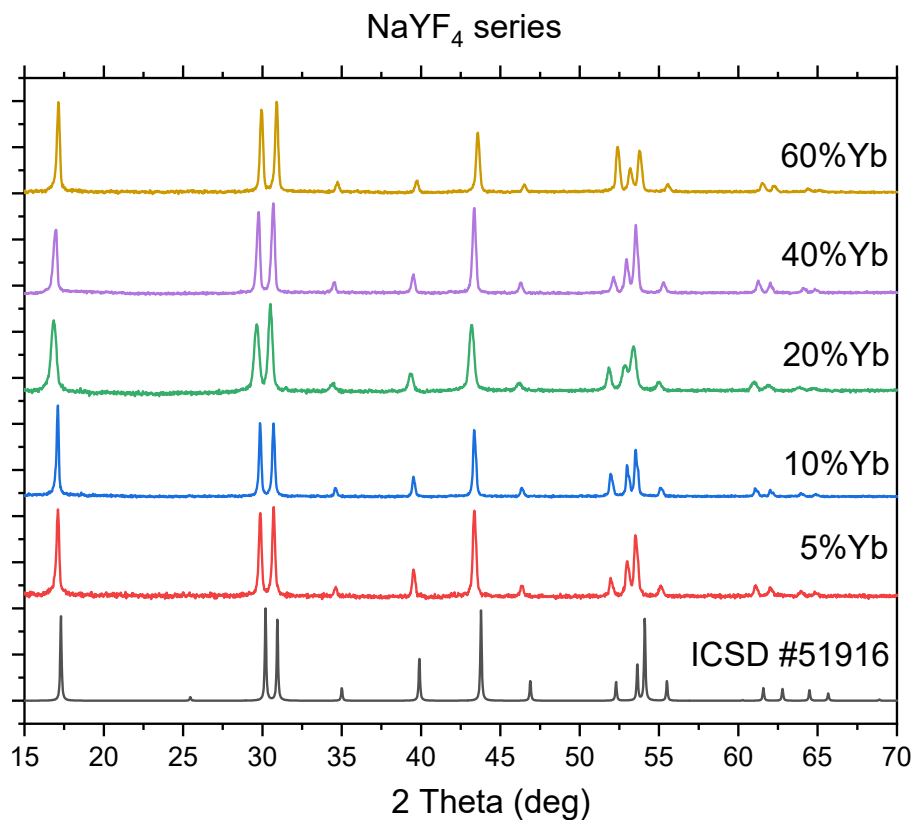


Figure S5: PXRD pattern of β -NaYF₄:Pr³⁺/Yb³⁺ series with a fixed amount of Pr and different Yb³⁺ concentrations (5, 10, 20, 40, 60 mol%). All PXRD patterns could be ascribed to the formation of the β -NaYF₄ host (ICSD # 51916).

Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) was used to determine the exact amount of selected lanthanide elements. The content of Yb³⁺ and Pr³⁺ were determined (in mg/kg) after wet digestion method, followed by the standard ICP-OES method (%RSD for n=3), taking into account the blank. The raw data was further calculated to obtained mol% ratio OF the Yb³⁺ and Pr³⁺ as Yb³⁺/Pr³⁺ and summarise as below (**Table S3**):

Table S3: ICP-OES of Yb³⁺ and Pr³⁺ in both NaYF₄ and LiYF₄ NC series.

NaYF ₄ (mol% of Yb ³⁺ /Pr ³⁺)		LiYF ₄ (mol% of Yb ³⁺ /Pr ³⁺)	
Experimental	Theoretical	Experimental	Theoretical
5.23	5.0	1.19	1.0
11.26	10.0	2.62	2.5
18.56	20.0	5.03	5.0
46.11	40.0	11.04	10.0
52.55	60.0		

SI 3 Photoluminescence properties

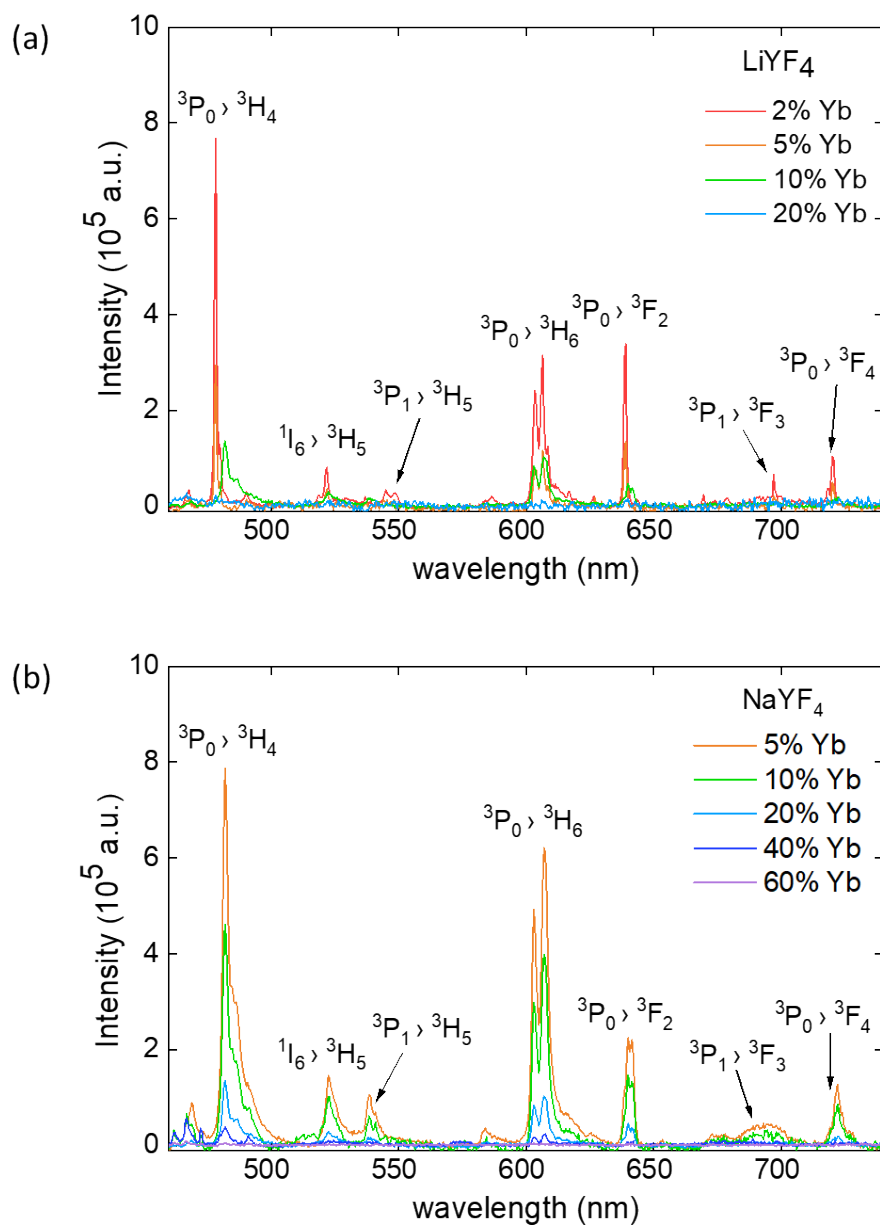


Figure S6: Stokes PL emission in the visible range for a) $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ and b) $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs with a range of different Yb^{3+} concentrations in CHCl_3 under 447 nm excitation.

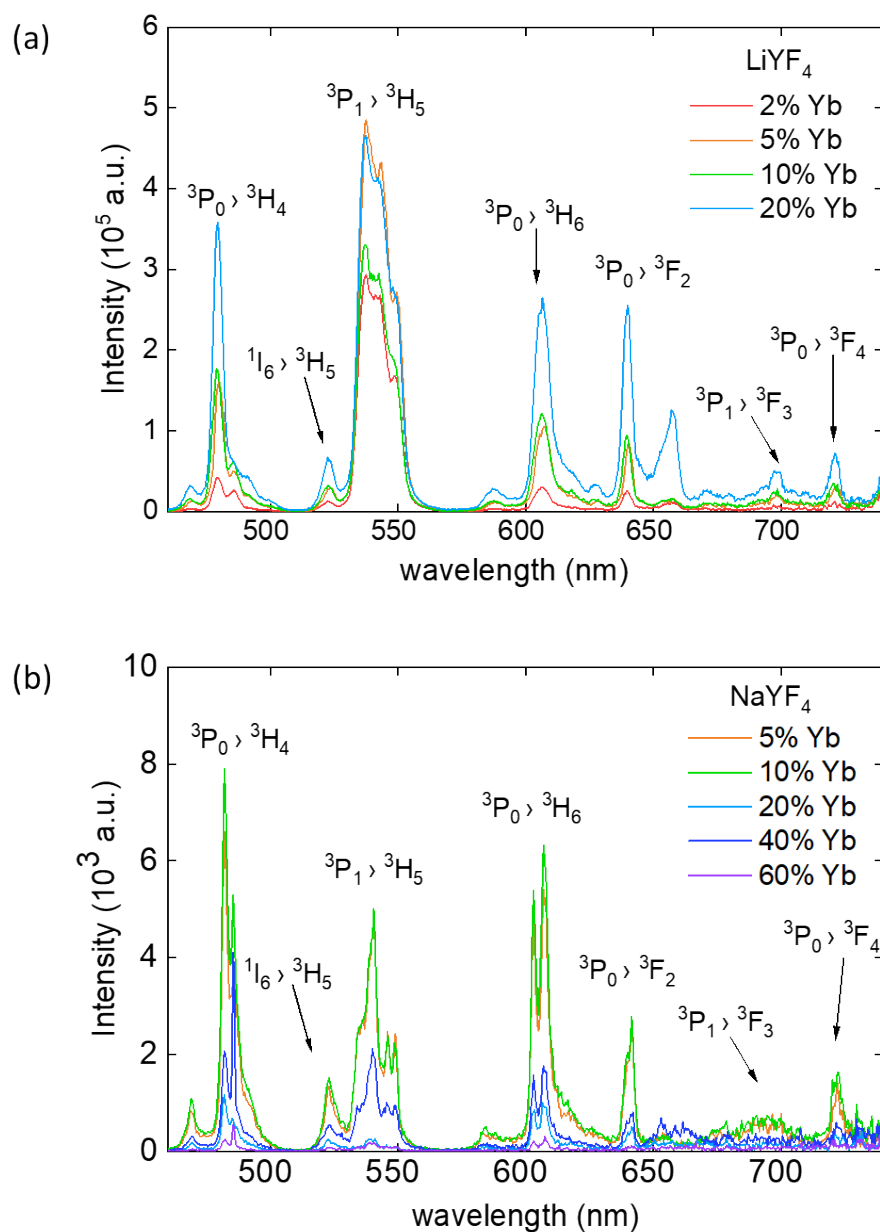


Figure S7: Upconversion PL emission of a) LiYF₄:Pr³⁺/Yb³⁺ and b) NaYF₄:Pr³⁺/Yb³⁺ NCs with different Yb³⁺ concentrations in visible range in CHCl₃ under 980 nm excitation.

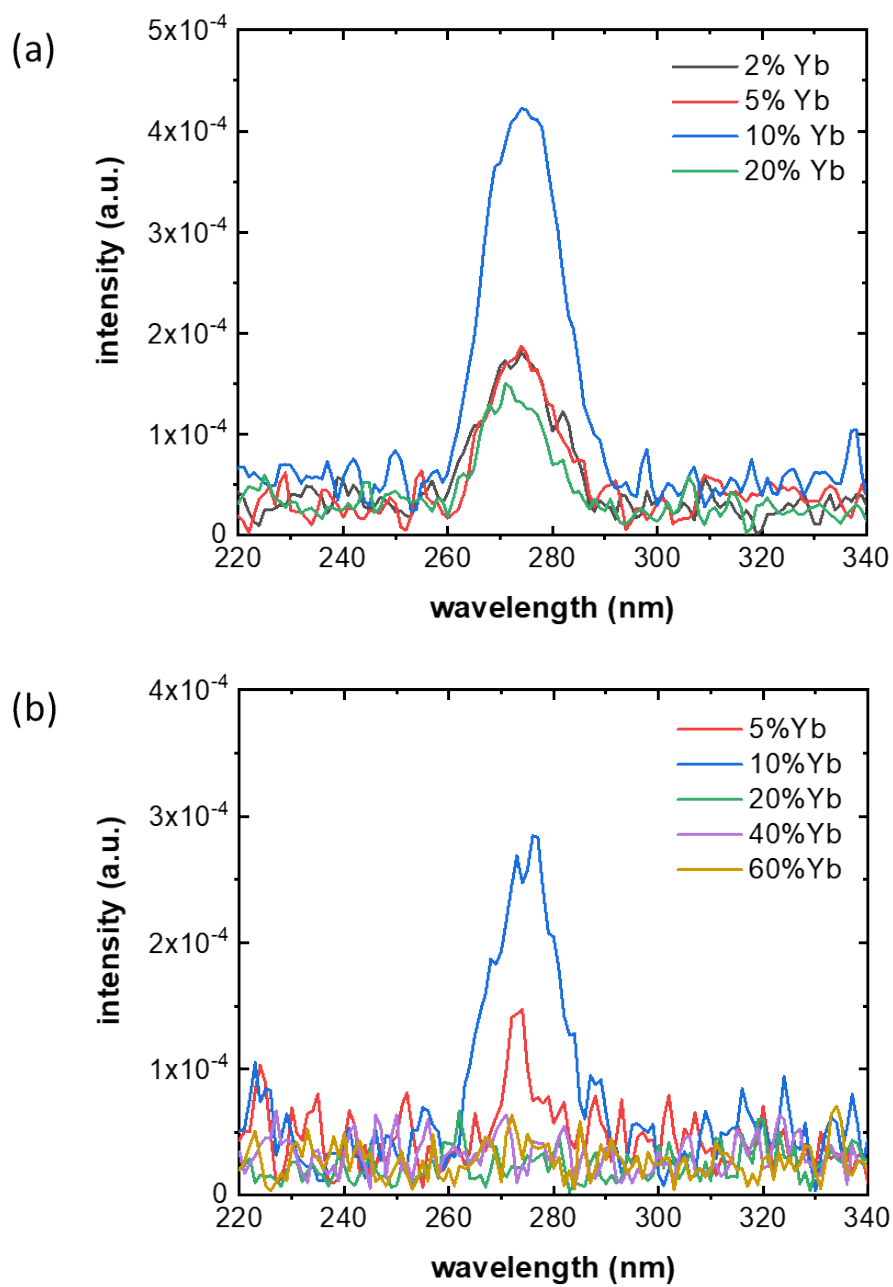


Figure S8: UVC PL emission spectra of $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ and $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NC in powder form with different Yb^{3+} concentrations under 447 nm laser excitation.

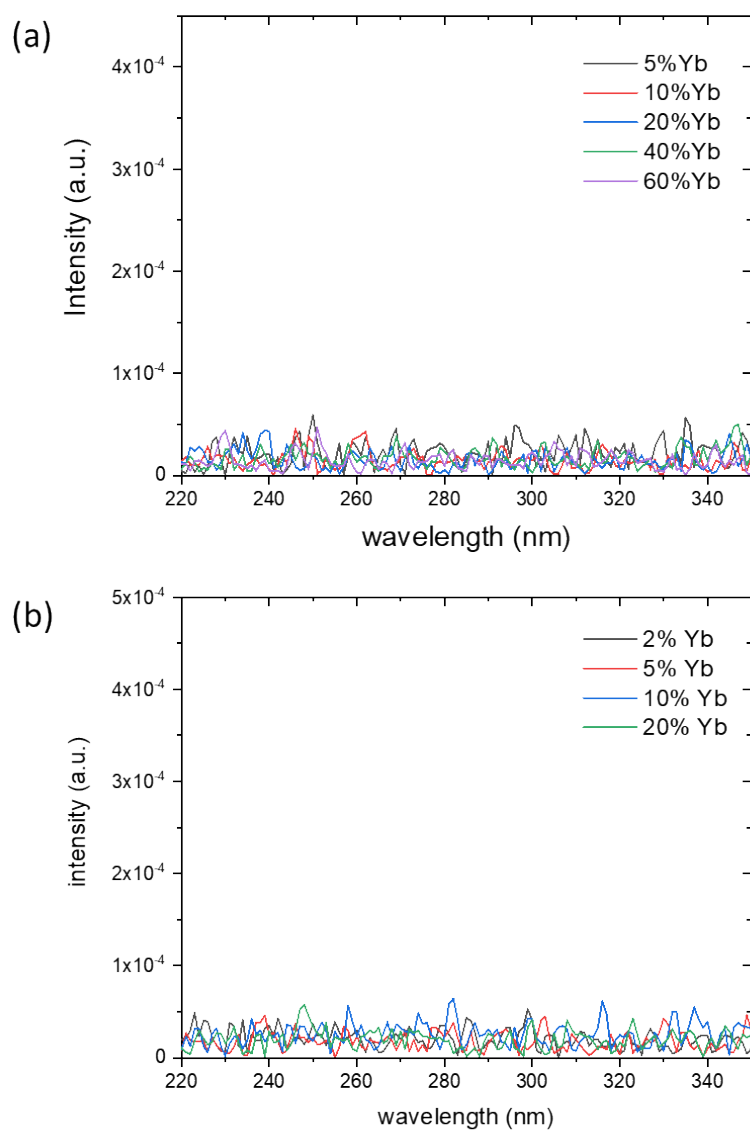


Figure S9: UVC PL emission spectra of $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ and $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs in powder form with different Yb^{3+} concentrations under 980 nm laser excitation.

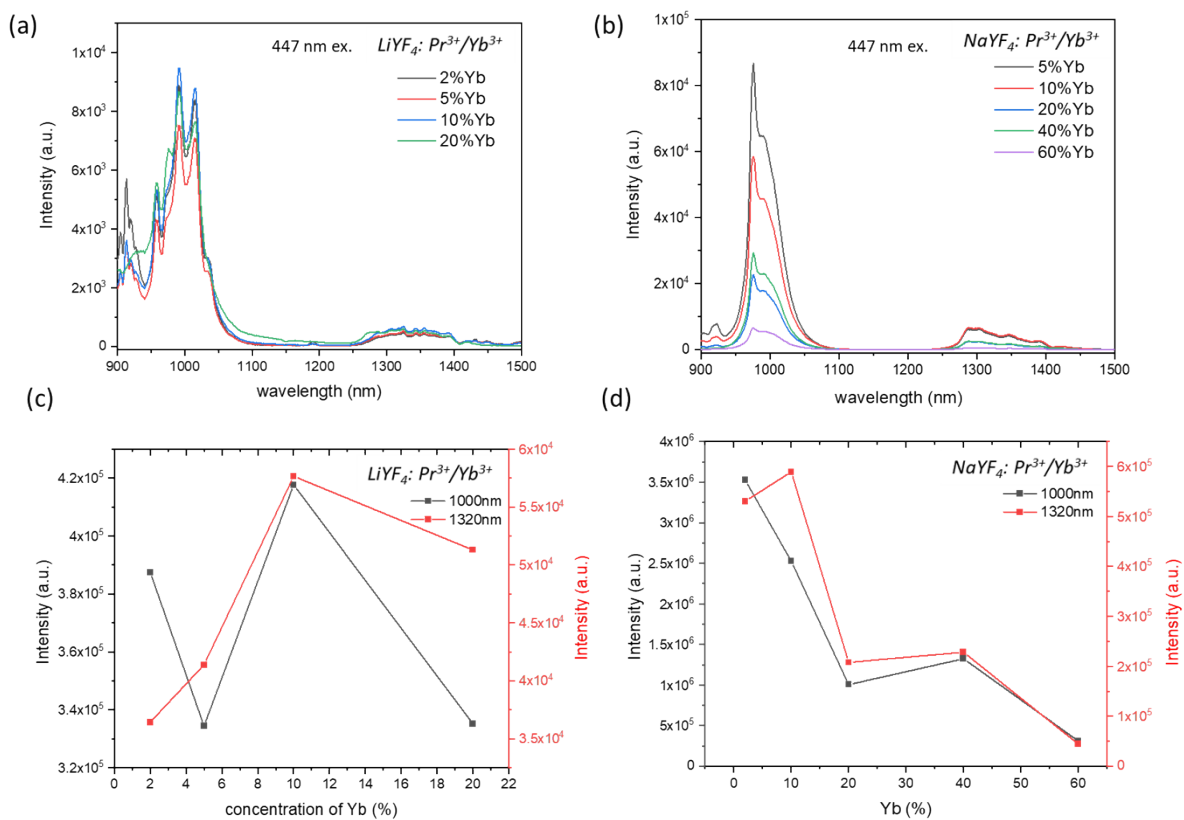


Figure S10: NIR PL emission of LiYF₄:Pr³⁺/Yb³⁺ and β-NaYF₄:Pr³⁺/Yb³⁺ NCs with different Yb³⁺ concentrations in CHCl₃ under 447 nm laser excitation; a,b) emission spectra and c,d) integrated areas of emission intensity at 1000 nm (black) and 1320 nm (red).

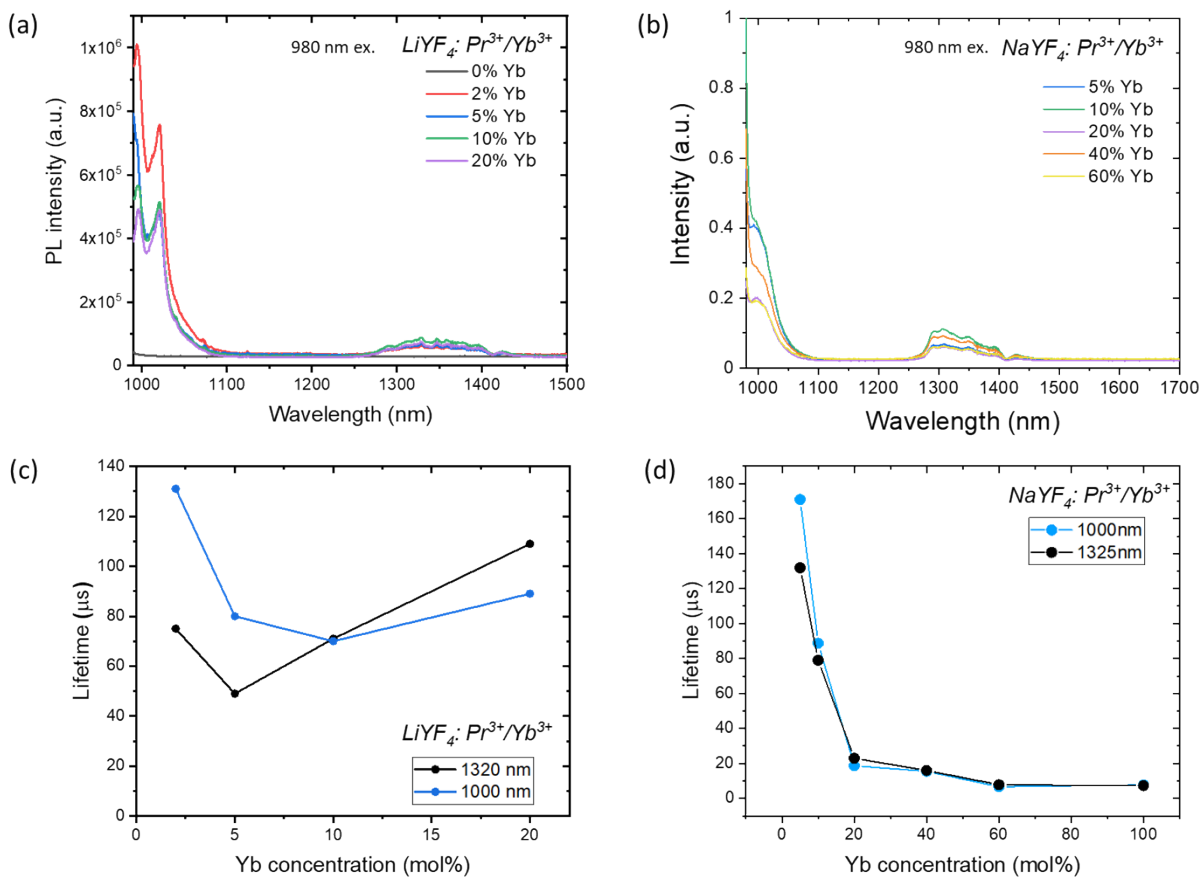


Figure S11: NIR PL emission for a) $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ and b) $\beta\text{-NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs and lifetime measurements at ca. 1000 nm (Yb^{3+}) and 1320 nm (Pr^{3+}) for c) $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs and d) $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs with different Yb^{3+} concentrations in CHCl_3 under 980 nm excitation.

SI 4 DNA denaturation demonstration

UV-Vis absorption study

50 $\mu\text{g}/\text{mL}$ dsDNA solution was prepared for the denaturation study. 0.5 mL dsDNA solution was added to a quartz cuvette and placed in front of the glass slide (with or without NC powder). The samples were irradiated by the collimated 447 nm laser beam at $\sim 11 \text{ W}/\text{cm}^2$ power density). The UV-Vis spectra in 200-350 nm range were recorded before and after the irradiation.

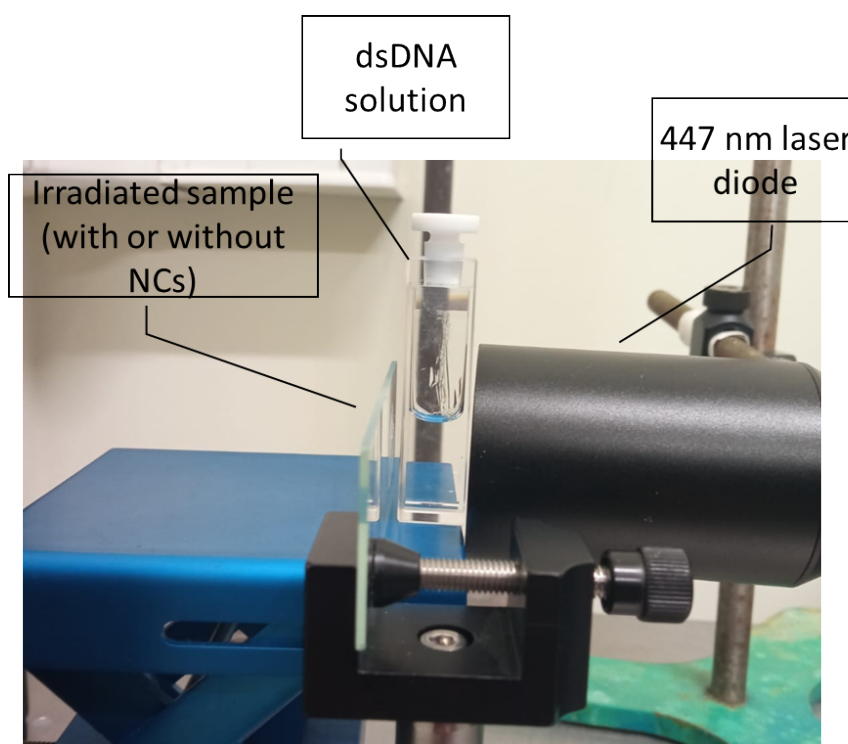


Figure S12: Setup of DNA denaturation experiment. The dsDNA denaturation was conducted by depositing the corresponding $\text{LiYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ or $\text{NaYF}_4:\text{Pr}^{3+}/\text{Yb}^{3+}$ NCs on a glass slide and placing it behind the cuvette with dsDNA solution, the CW 447 nm laser diode was placed in front of the cuvette with ds DNA solution.

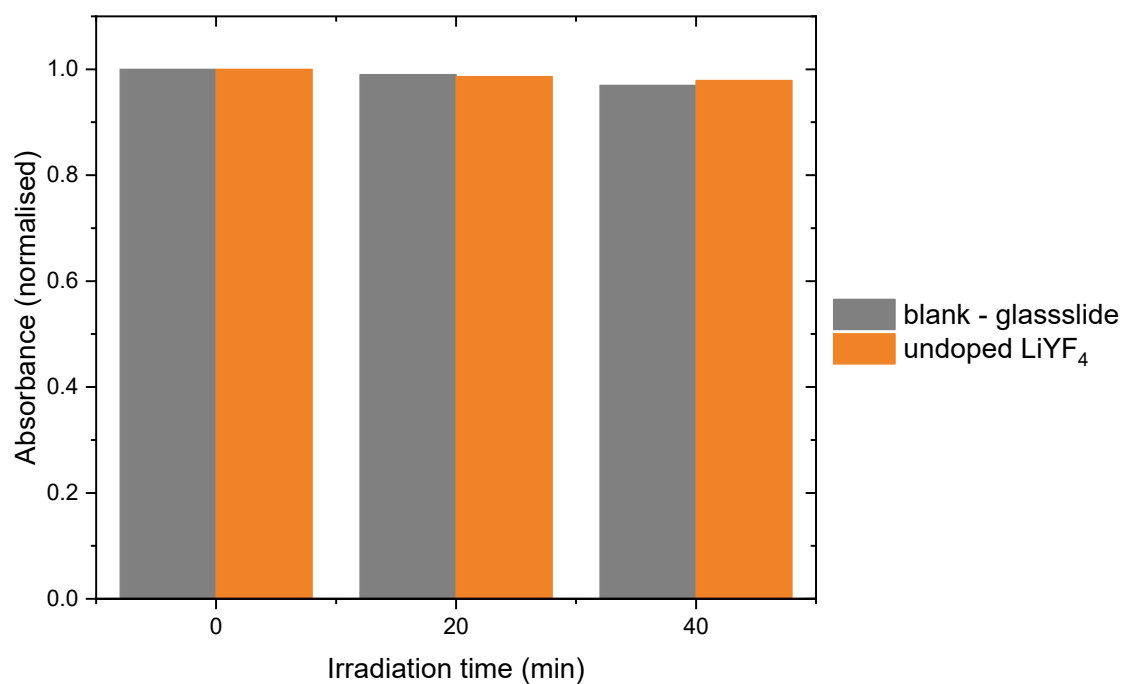


Figure S13: Absorbance of salmon sperm dsDNA solution after 20 and 40 min irradiation with 447 nm laser at ~ 11 W/cm².

Fluorometric analysis of DNA unwinding (FADU) assay

The assay solution was prepared according to standard protocol described elsewhere.⁴ In general, 20 μL of the 50 $\mu\text{g}/\text{mL}$ dsDNA solution and the corresponding diluted dsDNA solution or irradiated dsDNA solution was added to 2 mL assay solution. The mixture was allowed to react for 2-3 min and the fluorescent emission from 380 to 680 nm were measured under 365 nm excitation wavelength. Titration was performed in order to estimate the optimal amount of dsDNA solution that would saturate the dye (Figure S13). It is found that the optimal amount of dsDNA solution (concentration: 50 $\mu\text{g}/\text{mL}$) selected is 250 μL to avoid over saturation of the dye.

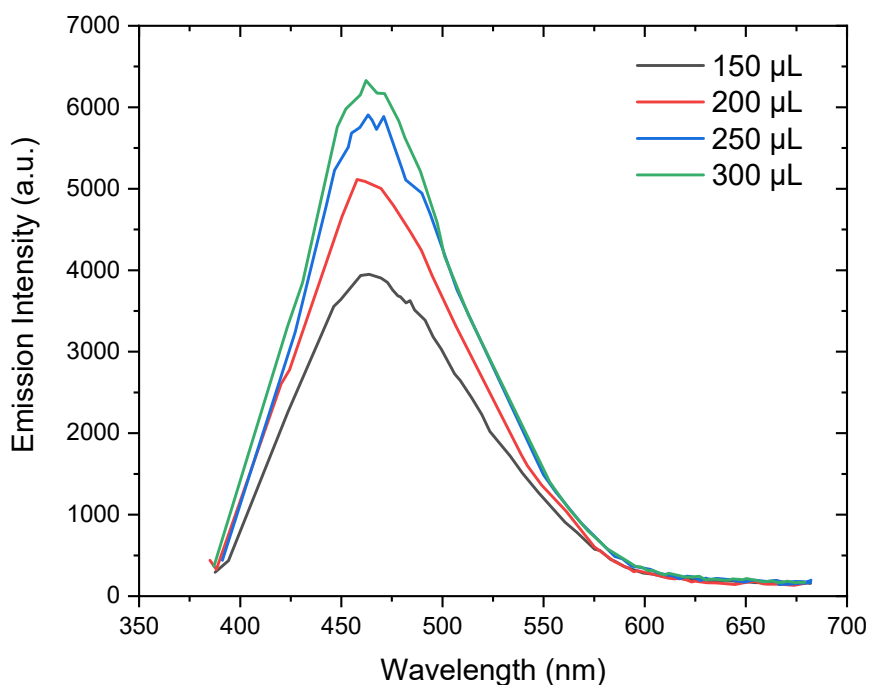


Figure S14: Fluorescence spectra of Hoechst 32258 dye with addition of a different amount of dsDNA solution (150, 220, 250 and 300 μL).

Next, a calibration experiments with different concentration of dsDNA were performed and the obtained calibration graph (Figure S14) is used to estimate the dsDNA amount after irradiation.

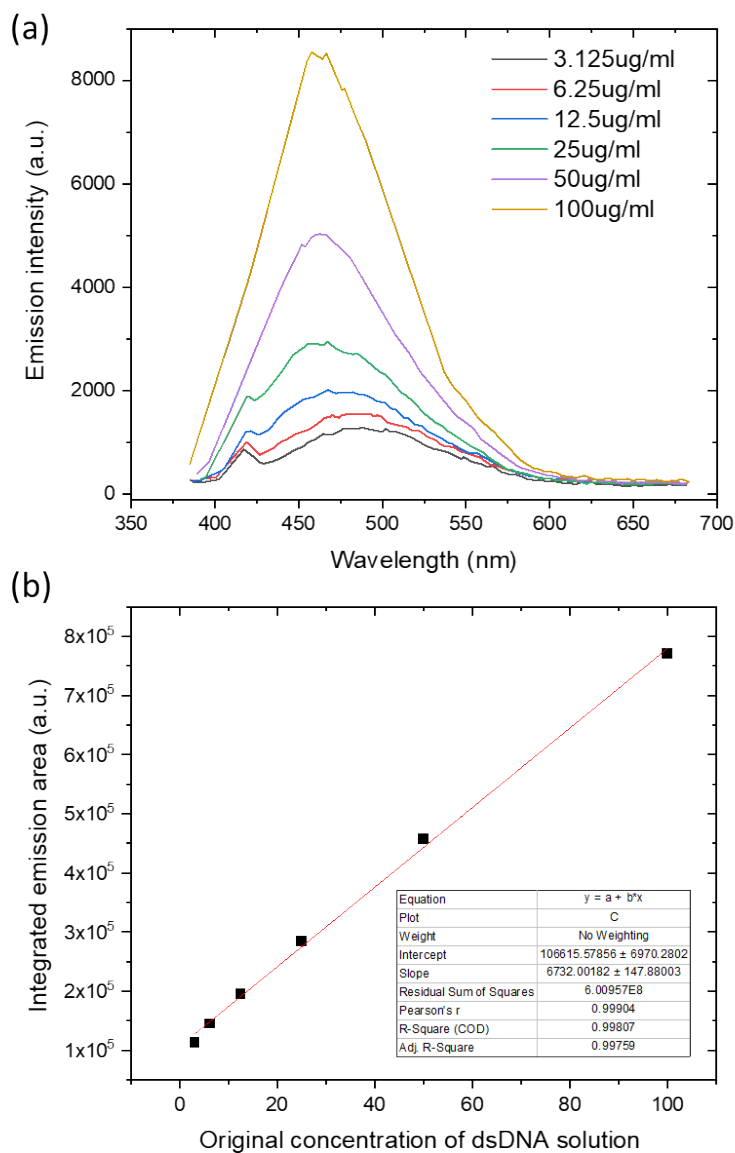


Figure S15: Fluorescence spectra of Hoechst 33258 solution with different dsDNA concentrations (a) and the corresponding calibration curve (b).

For the FADU assay study, 250 μL of dsDNA solution at each analysis (before and after UVC irradiation) are analyzed in order to obtain qualitative and comparative data. Each measurement was also repeated at least 2 times.

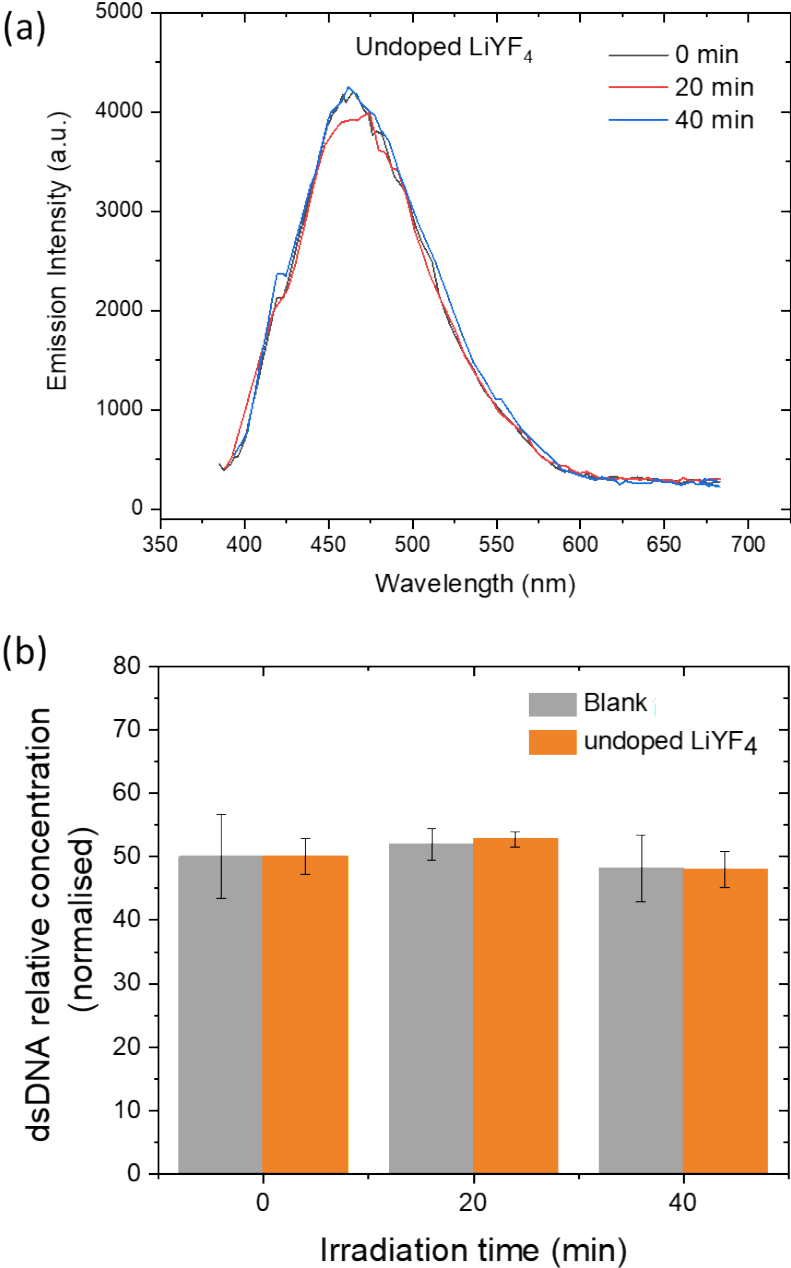


Figure S16: dsDNA relative concentration of salmon sperm dsDNA solution determined by calibration curve after 20 and 40 min irradiation of blank glass and undoped LiYF₄ NCs with 447 nm laser beam (at 11 W/cm²).

SI 5 NIR-II PL imaging

Short Wave Infrared (SWIR) imaging camera (Xeva-1.7-320, Xenics, Belgium) equipped with focusing optics (TECM55MPW, Computar, USA) was used to image a NIR-II photoluminescence from the samples irradiated by a defocused beam from 980 nm laser diode (QSP-975-10, QPhotonics) with power density of 100 mW/cm².

SI 6 References

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