Supporting Information

Er^{3+}-to-dye energy transfer in DNA-coated core and core/shell/shell upconverting nanoparticles with 980 nm and 808 nm excitation of Yb^{3+} and Nd^{3+}

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Experimental procedures

- ***Calculation of the spectral overlap integral (J)***

The overlap between the UCNP emission (donor) and Cy3.5 absorption (acceptor) was quantified with the spectral overlap integral (*J*), which was calculated by using the following equation:

$$J = \sum \varepsilon_A(\lambda) \cdot F_D(\lambda) \cdot \lambda^4 \cdot d\lambda \qquad \text{Equation S1}$$

where ε_A is the molar extinction coefficient spectrum of the Cy3.5-ssDNA acceptor (ε_{590nm} = 115,360 $cm^{-1} \cdot M^{-1}$), F_D is the donor emission spectrum normalized to its area and λ is the wavelength of light. The spectra in Figure 2 (in the manuscript) were used for the calculations. When taking into account only the green UCL bands (shown in Figure 2 in the manuscript) for normalization, the overlap integral was $J = 3.1 \pm 0.1 \times 10^{15}\ \lambda^4 M^{-1} cm^{-1}$. When taking into account both the green and red UCL bands (shown in Figure 1 in the manuscript) for normalization, the overlap integral was $J = 2.6 \pm 0.5 \times 10^{15}\ \lambda^4 M^{-1} cm^{-1}$.

- ***Fitting of UCL kinetics***

For 980 nm excitation, the rise and decay times (τ_R and τ_D, respectively) were determined by fitting the data to the following equation:

$$y = y_0 + A \cdot \left(1 - e^{-\frac{t-t_0}{\tau_R}}\right) \cdot e^{-\frac{t-t_0}{\tau_D}} \qquad \text{Equation S2}$$

For 808 nm excitation, the rise and decay times were determined separately because the signal intensities were not sufficiently high to perform adequate fitting with Equation S2. The rise and decay part of the UCNPs emission kinetic profiles were fit to monoexponential growth (Equation S3) and decay functions (equation S4), respectively.

$$y = y_0 + A \cdot e^{t/\tau_R} \qquad \text{Equation S3}$$

$$y = y_0 + A \cdot e^{-t/\tau_D} \qquad \text{Equation S4}$$

- ***Estimation of UCNP concentrations***

UCNP concentrations in number of nanoparticles per mL, were estimated following a previously described procedure.[1] The nanoparticles were approximated to a sphere and their volume (V_{UCNP}) was calculated using Equation S5.

$$V_{UCNP} = \frac{4}{3} \cdot \pi \cdot \left(\frac{d}{2}\right)^3 \qquad \text{Equation S5}$$

where *d* was the diameter of the UCNP. The volume of one UCNP was 1.0 x 10^4 nm^3 for c-UCNPs and 1.6 x 10^4 nm^3 for css-UCNPs (of which 9.2 x 10^3 nm^3 corresponds to the core, 2.3 x 10^3 nm^3 to the Nd-shell and 4.1 x 10^3 nm^3 to the inert shell).
Taking into account the hexagonal lattice parameters[1] a_h (5.96 Å) and c_h (3.51 Å), the volume of a hexagonal unit cell (V_{unit_cell}) was calculated by:

$$V_{unit\_cell} = \frac{2\sqrt{3}}{4} \cdot a_h^2 \cdot c_h \qquad \text{Equation S6}$$

Hence, the number of unit cells per UCNP (N_{unit_cell}) was given by:

$$N_{unit\_cell} = {}^{V_{UCNP}}/_{V_{unit\_cell}} \qquad \text{Equation S7}$$

Therefore, a spherical UCNP contains ~1.0 x 10^5 unit cells per UCNP for c-UCNPs and ~1.4 x 10^5 for css-UCNPs (with ~8.5 x 10^4 unit cells in the core, ~2.1 x 10^4 unit cells in the Nd shell and ~3.8 x 10^4 unit cells in the inert shell).
The atomic weight of a single hexagonal cell (*AW*) of undoped crystals was described by:

$$AW_{undoped} = 1.5 \cdot AW_{Na} + 1.5 \cdot AW_Y + 6 \cdot AW_F \qquad \text{Equation S8}$$

whereas for doped crystals, an additive factor (*af*) was defined as a numeric value between 0 and 1, corresponding to 0% and 100% yttrium substitution, respectively. In our case, the *af* value was 0.22 for the β-$NaYF_4$:Yb (20%), Er(2%) core, and 0.20 for β-$NaYF_4$:Yb (20%) Nd-doped shell. Thus, the equation to calculate the *AW* value for the UCNP core was determined by:

$$AW_{core} = 1.5 \cdot AW_{Na} + 6 \cdot AW_F + 1.5 \cdot (1 - 0.22) \cdot AW_Y + 1.5 \cdot (0.2) \cdot AW_{Yb} + 1.5 \cdot (0.02) \cdot AW_{Er} \qquad \text{Equation S9}$$

And for the Nd-doped shell was calculated by:

$$AW_{Nd\_doped} = 1.5 \cdot AW_{Na} + 6 \cdot AW_F + 1.5 \cdot (1 - 0.2) \cdot AW_Y + 1.5 \cdot (0.2) \cdot AW_{Nd} \qquad \text{Equation S10}$$

Subsequently, molecular weight of c-UCNPs (*MW*) was estimated assuming that the unit cells were distributed uniformly along the UCNP by using the following equation:

$$MW = AW_{core} \cdot N_{unit\_cell} \qquad \text{Equation S11}$$

obtaining a value of 3.0 x 10^7 g/mol (30 MDa). To estimate the molecular weight of css-UCNPs, three different partial MW were calculated: β-$NaYF_4$:Yb (20%), Er(2%) core, the β-$NaYF_4$:Yb (20%) Nd-doped shell, and the undoped shell. The total molecular weight of css-UCNPs was estimated by adding up these three values, being 4.3 x 10^7 g/mol (43 MDa).
With the estimated *MW* values, we converted the mg/mL concentration into mmol/mL and subsequently with NP/mL by using Avogadro´s constant.

- ***Estimation of the amount of ss-DNA per NP***

To estimate the number of ss-DNA attached on the surface, we analyzed the supernatants and washed off solvent (water) for all samples by absorption spectroscopy. The molar extinction coefficient (ε) of Cy3.5-ssDNA (ε_{590nm} = 115,360 $cm^{-1} \cdot M^{-1}$) and ss-DNA (ε_{257nm} = 209,600 $cm^{-1} \cdot M^{-1}$) were previously calculated by measuring the absorption of water solutions with known increasing concentrations of Cy3.5-ssDNA. The concentrations in the supernatants were calculated by using ε and the corresponding absorption maxima. The anchored ligand amount was calculated by subtracting the amount of ss-DNA in the supernatants from the amount of ss-DNA added in the reaction.

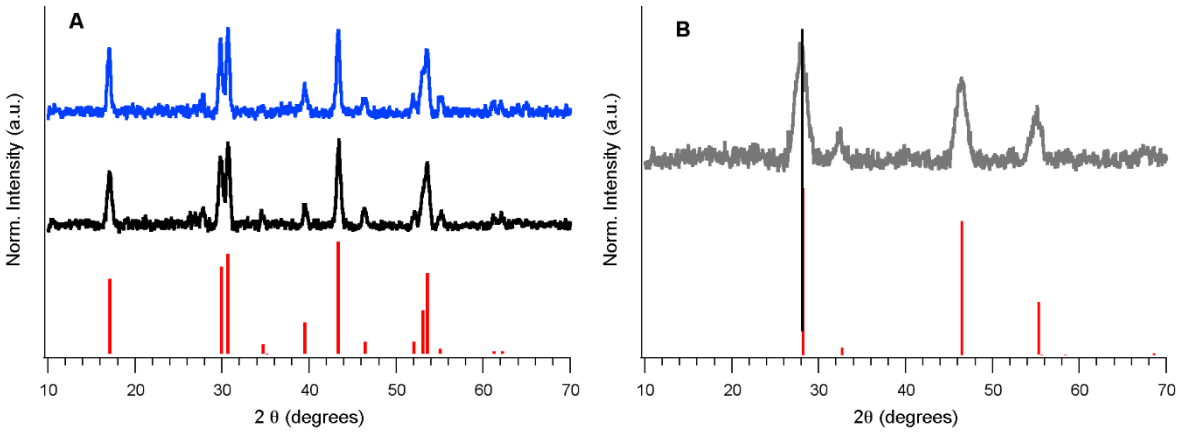

Figure S1- Powder X-ray diffraction (XRD) patterns of (A) c-UCNPs (black line), css-UCNPs (blue line) and reference β-$NaYF_4$ hexagonal phase (red bar) (JCPDS no. 28-1192); (B) s-UCNPs (gray line) and reference α-$NaYF_4$ cubic phase (red bar) (JCPDS no. 77-2042).

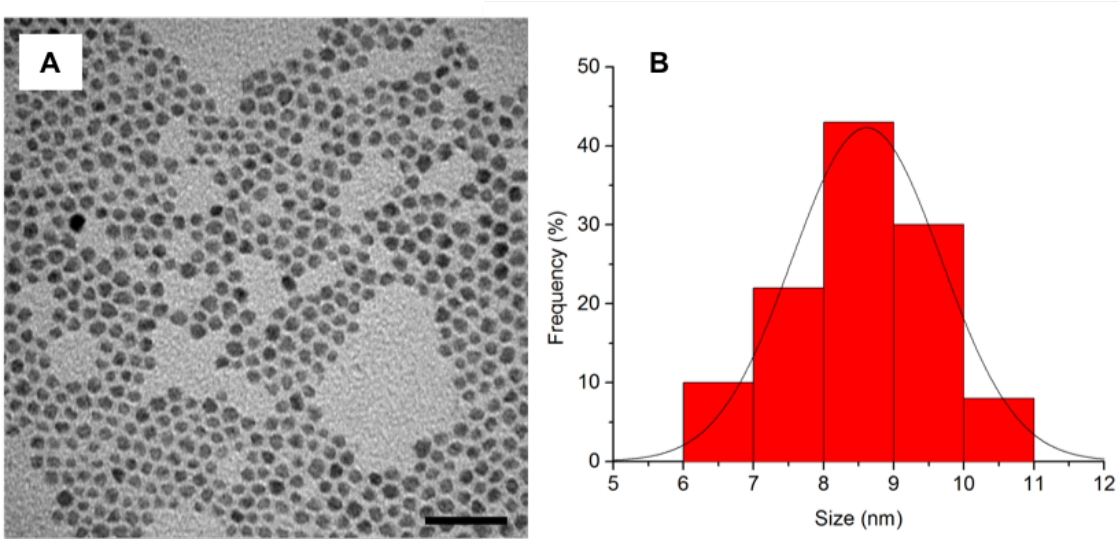

Figure S2- Transmission electron micrograph of (A) s-UCNPs, and (B) histogram of the particle size distribution. Scale bar: 50 nm.

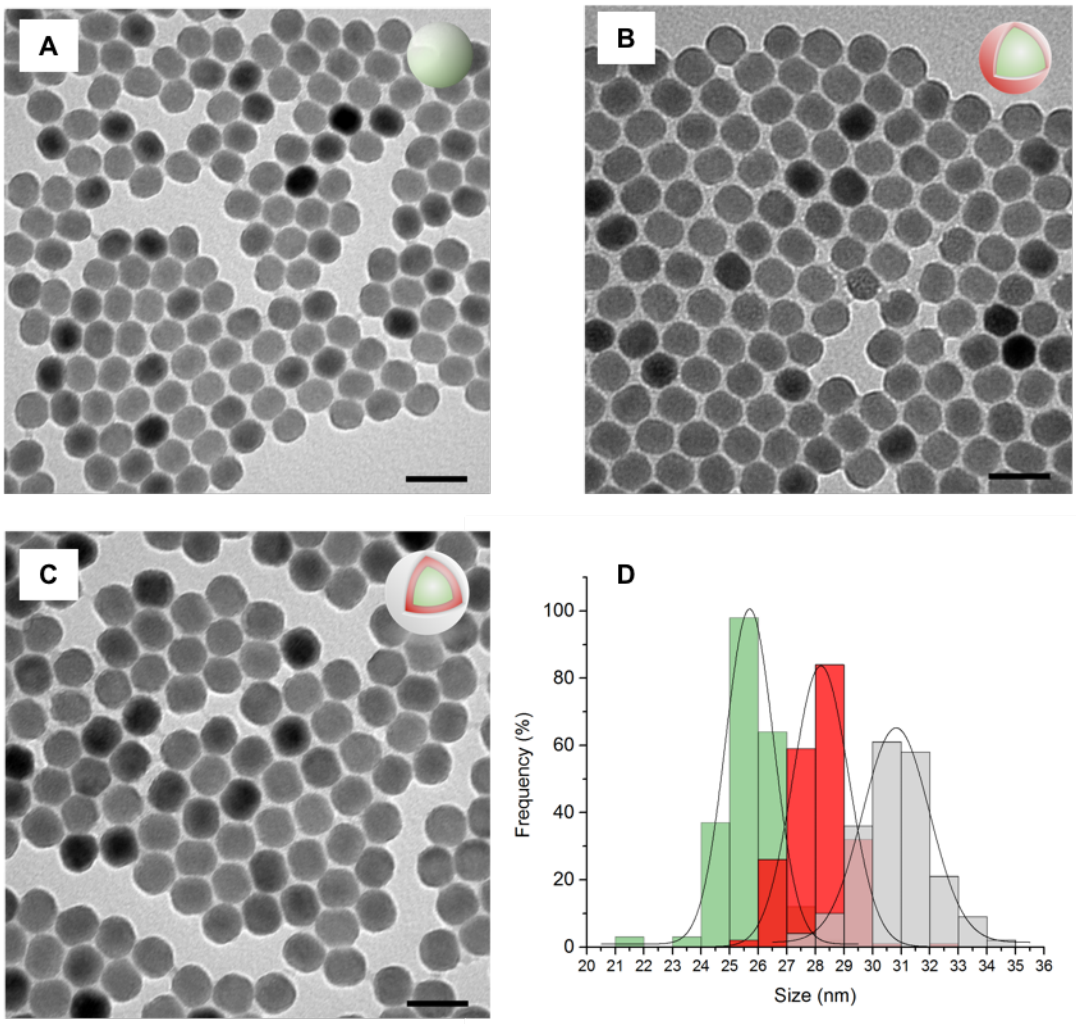

Figure S3- Transmission electron micrographs of (A) c-UCNPs, (B) cs-UCNPs, and (C) css-UCNPs. Scale bars: 50 nm. (D) Histograms of the particle size distributions of the nanoparticles as determined by TEM: c-UCNPs (green), cs-UCNPs (red), and css-UCNPs (gray).

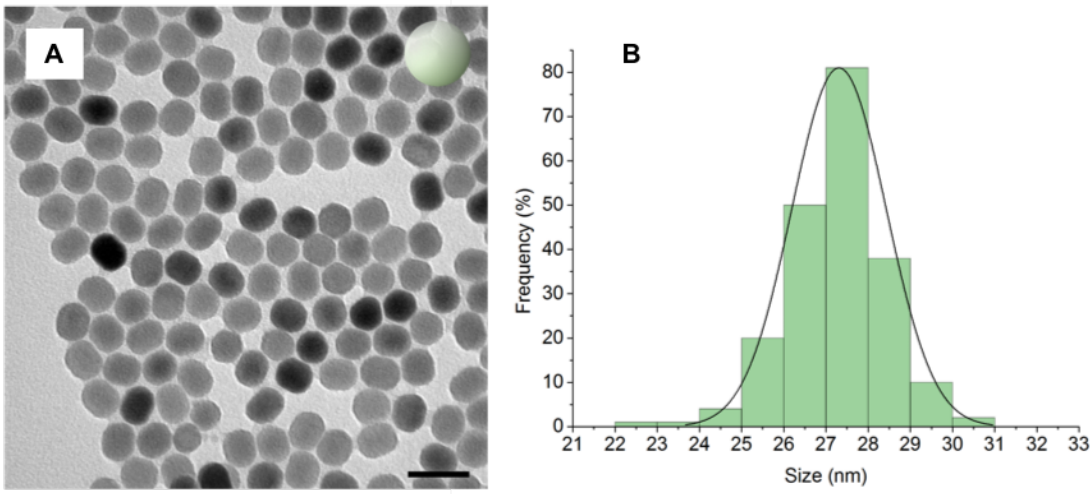

Figure S4- Transmission electron micrograph of (A) c-UCNPs. Scale bar: 50 nm. (B) Histogram of the particle size distribution of the nanoparticles as determined by TEM.

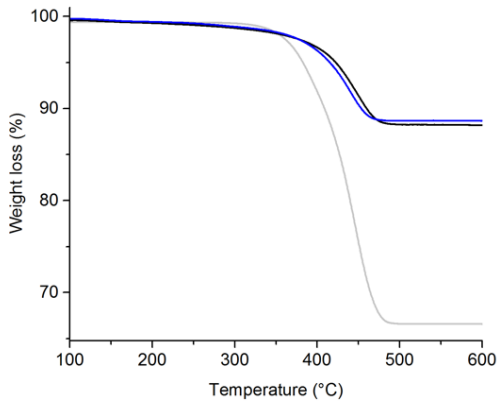

Figure S5- Thermogravimetric analysis (TGA) of undoped s-UCNPs (light gray line), c-UCNPs (black line) and css-UCNPs (blue line).

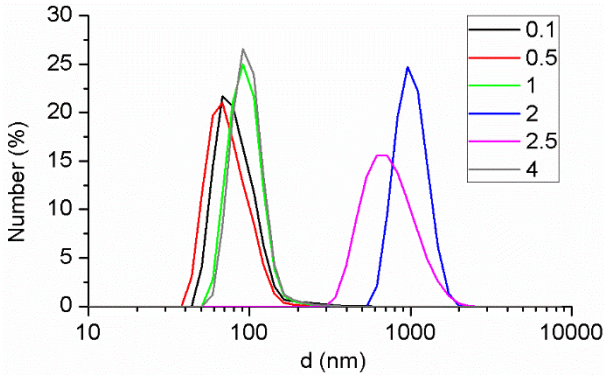

Figure S6- Hydrodynamic diameter (number-weighted distribution) of the different Cy3.5-ssDNA-c-UCNP nanohybrids in water (1 mg/mL): 0.1 (black line), 0.5 (red line), 1 (green line), 2 (blue line), 2.5 (light pink line) and 4 (gray line) nmol/mg of Cy3.5-ssDNA per c-UCNP ratios.

Table S1- Hydrodynamic diameter and corresponding polidispersity index (PDI) values determined by DLS of the nanohybrids in water for each Cy3.5-ssDNA:c-UCNP nominal ratio.

ssDNA:c-UCNP ratio (nmol/mg)	*d (nm)*	*PDI*
0.1	86.2 ± 0.8	0.37 ± 0.04
0.5	75 ± 6	0.23 ± 0.02
1	92 ± 8	0.26 ± 0.03
2	987 ± 164	0.35 ± 0.05
2.5	594 ± 394	0.26 ± 0.01
4	98 ± 4	0.11 ± 0.02

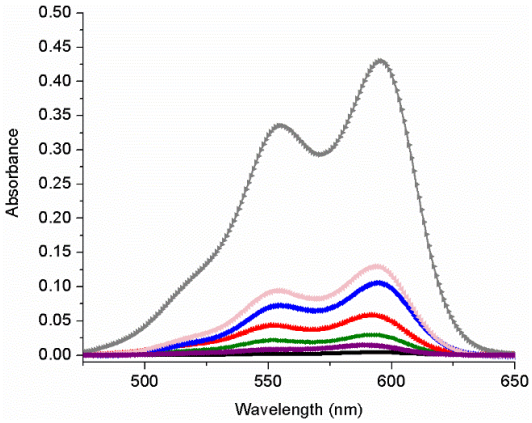

Figure S7- Absorption spectra of c-UCNP-DNA nanohybrids with different Cy3.5-ssDNA:UCNP ratios in water (1 mg/mL). Cy3.5-ssDNA:UCNP nominal ratio equal to 0.1 (black line), 0.5 (red line), 1.0 (green line), 2.0 (blue line), 2.5 (light pink line) and 4.0 (gray line) nmol/mg, respectively. The absorption spectrum of Cy3.5-ssDNA is also reported as a reference (purple line).

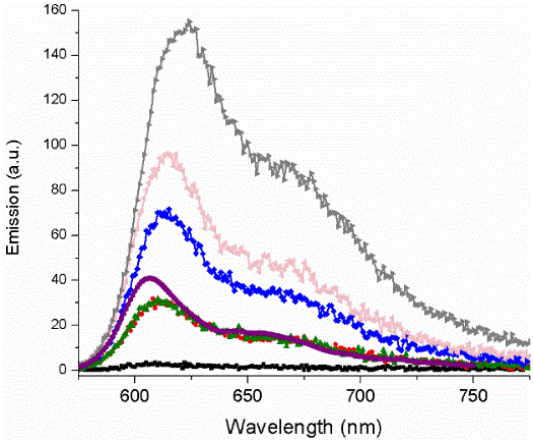

Figure S8- PL emission spectra (λ_{exc} = 552 nm) of c-UCNP-DNA nanohybrids with different Cy3.5-ssDNA:c-UCNP ratios in water (1 mg/mL). Cy3.5-ssDNA:c-UCNP ratio equal to 0.1 (black line), 0.5 (red line), 1.0 (green line), 2.0 (blue line), 2.5 (light pink line) and 4.0 (gray line) nmol/mg, respectively. The PL emission spectrum of Cy3.5-ssDNA is also reported as a reference (purple line).

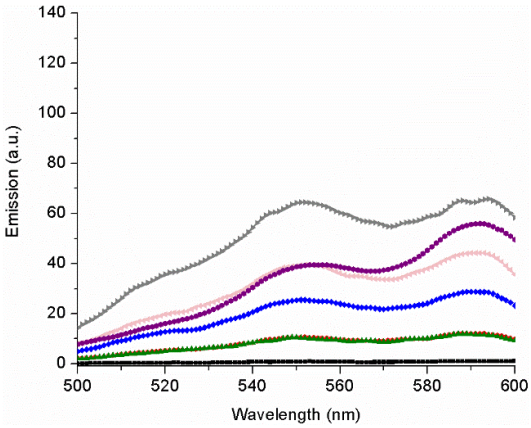

Figure S9- PL excitation spectrum (λ_{em} = 614 nm) of c-UCNP-DNA nanohybrids with different Cy3.5-ssDNA:c-UCNP ratios in water (1 mg/mL. Cy3.5-ssDNA:c-UCNP ratio equal to 0.1 (black line), 0.5 (red line), 1.0 (green line), 2.0 (blue line), 2.5 (light pink line) and 4.0 (gray line) nmol/mg, respectively. The PL excitation spectrum of Cy3.5-ssDNA is also reported as a reference (purple line).

Table S2- DLS measurements and ζ-potential for the c-UCNPs and css-UCNPs capped with unlabelled and Cy3.5-labelled ssDNA, respectively. In both cases the DNA:UCNP ratio is kept at 4 nmol/mg.

Sample	*d (nm)*	*PDI*	*ζ (mV)*
c-UCNPs@ssDNA	176 ± 4	0.17 ± 0.04	-10 ± 1
c-UCNPs@Cy3.5-ssDNA	136 ± 4	0.18 ± 0.01	-12.7 ± 0.9
css-UCNPs@ssDNA	187 ± 5	0.14 ± 0.01	-7 ± 3
css-UCNPs@Cy3.5-ssDNA	112 ± 8	0.18 ± 0.05	-3.5 ± 1.0

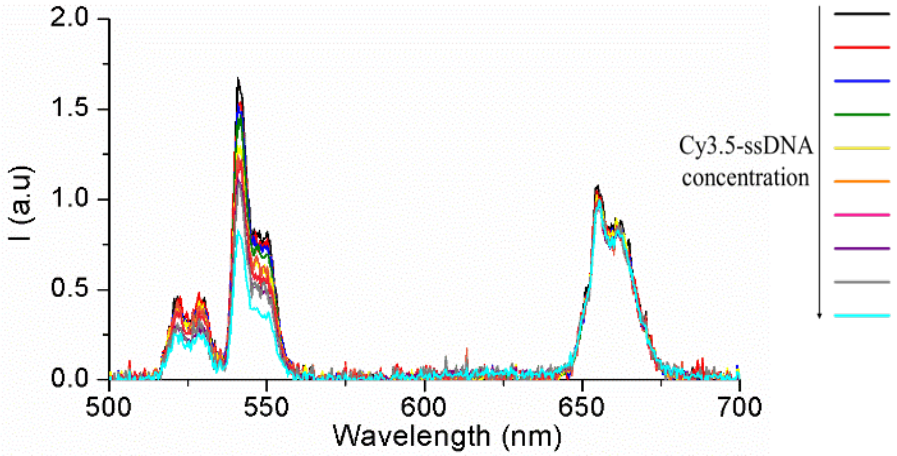

Figure S10- PL emission spectra for different amounts Cy3.5-ssDNA/ssDNA under 980 nm excitation in water (10 mg/mL) for c-UCNP-DNA nanohybrids.

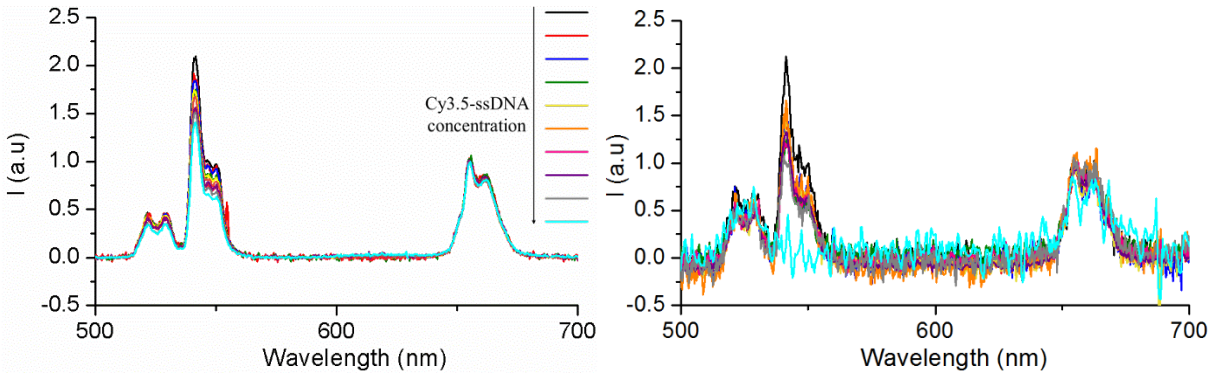

Figure S11- PL emission spectra for different amounts Cy3.5-ssDNA/ssDNA under 980 nm (left) and 808 nm (right) excitation in water (10 mg/mL) for css-UCNP-DNA nanohybrids. Due to the significantly lower PL intensities upon 808 nm excitation, the spectra on the right are much noisier.

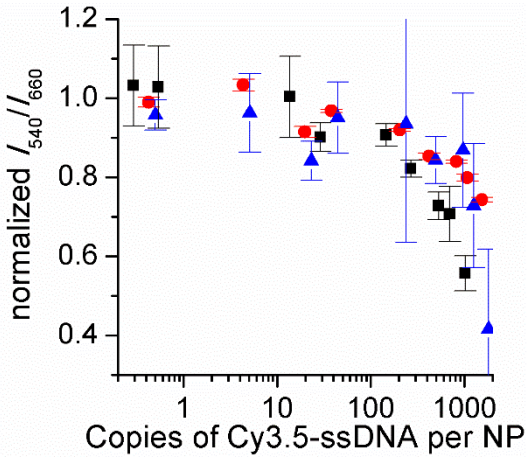

Figure S12- Cy3.5-ssDNA copy numbers dependent PL intensity ratios (I_{540}/I_{660} - normalized to unity for samples without Cy3.5-ssDNA) of c-UCNP (black) and css-UCNP (red) upon excitation at 980 nm and of css-UCNP upon 808 nm excitation (blue).

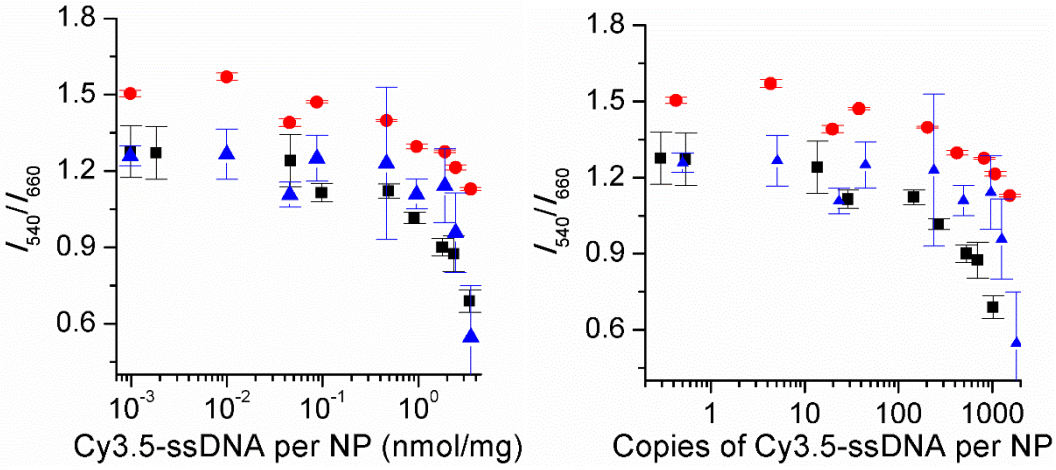

Figure S13- PL intensity ratios (I_{540}/I_{660}) of c-UCNP (black) and css-UCNP (red) upon excitation at 980 nm and of css-UCNP upon 808 nm excitation (blue) as function of the Cy3.5-DNA concentration (left) and of the copy numbers of Cy3.5-ssDNA (right)

Table S3. Decay times of the different UCNP-DNA FRET systems in the UCNP detection channel (542/20 nm – cf. Figure S14) and the Cy3.5 detection channel (607/10 nm – cf. Figure S15). Decay times were determined by using Equations S2 (for 980 nm excitation) and S4 (for 808 nm excitation).

	Cy3.5-ssDNA/ssDNA- ***c-UCNP (980 nm)***		*Cy3.5-ssDNA/ssDNA-* ***css-UCNP (980 nm)***		*Cy3.5-ssDNA/ssDNA-* ***css-UCNP (808 nm)***	
Cy3.5-ssDNA (nmol/mg)	***UCNP 542/20 nm***	**Cy3.5 607/10 nm**	***UCNP 542/20 nm***	**Cy3.5 607/10 nm**	***UCNP 542/20 nm***	**Cy3.5 607/10 nm**
	τ_{DA} (*μs*)	τ_{AD} (*μs*)	τ_{DA} (*μs*)	τ_{AD} (*μs*)	τ_{DA} (*μs*)	τ_{AD} (*μs*)
0 (τ_{D0})	61.3 ± 0.1	8.2 ± 0.1	86.5 ± 0.4	6.2 ± 0.2	74.8 ± 1.0	4.6 ± 1.0
0.001	61.3 ± 0.2	11 ± 5	86.4 ± 0.2	5.6 ± 0.1	74.2 ± 1.0	5.6 ± 0.6
0.01	61.0 ± 0.3	24 ± 7	86.5 ± 0.5	6.6 ± 0.7	75.0 ± 0.7	5.7 ± 1.1
0.045	61.4 ± 0.3	33 ± 9	85.9 ± 0.1	13 ±2	74.1 ± 0.6	7.7 ± 1.2
0.09	60.6 ± 0.4	40 ± 6	84.6 ± 0.5	65 ± 10	72.9 ± 0.6	93 ± 17
0.47	59.7 ± 0.4	50 ± 3	83.1 ± 0.7	81 ± 5	73.4 ± 0.7	98 ± 14
0.97	57.8 ± 0.5	48.5 ± 0.7	81.8 ± 0.6	95 ± 4	71.5 ± 0.3	98 ± 9
1.90	54.7 ± 0.3	46.7 ± 0.5	77.3 ± 0.6	73.4 ± 0.3	68.6 ± 0.2	86 ± 5
2.47	51.3 ± 0.3	43.3 ± 0.9	73.8 ± 0.8	70.3 ± 1.2	65.5 ± 0.5	85 ± 5
3.54	49.1 ± 0.7	41.1 ± 1.0	70.9 ± 0.1	66.4 ± 0.9	63.9 ± 0.4	83 ± 3

Table S4. Rise times of the different UCNP-DNA FRET systems in the UCNP detection channel (542/20 nm – cf. Figure S14). Intensities in the Cy3.5 detection channel (607/10 nm – cf. Figure S15) were too low to detect and/or fit rise times. Rise times were determined by using Equations S2 (for 980 nm excitation) and S3 (for 808 nm excitation).

	Cy3.5-ssDNA/ssDNA- ***c-UCNP (980 nm)***	*Cy3.5-ssDNA/ssDNA-* ***css-UCNP (980 nm)***	*Cy3.5-ssDNA/ssDNA-* ***css-UCNP (808 nm)***
Cy3.5-ssDNA (nmol/mg)	τ_R (μs)	τ_R (μs)	τ_R (μs)
0	22.1 ± 0.2	74 ± 2	104 ± 2
0.001	21.01 ± 0.15	69.0 ± 0.5	97 ± 2
0.01	21.8 ± 0.3	70.2 ± 0.8	105 ± 3
0.045	21.6 ± 0.3	70.9 ± 0.7	103 ± 3
0.09	21.7 ± 0.2	71.97 ± 0.07	111 ± 2
0.47	22.0 ± 0.5	72 ± 2	109 ± 2
0.97	22.1 ± 0.2	73.2 ± 1.1	93 ± 1
1.90	22.62 ± 0.11	80 ± 2	102.3 ± 0.2
2.47	22.08 ± 0.07	78 ± 3	99 ± 2
3.54	22.9 ± 0.7	83.1 ± 0.6	108 ± 2

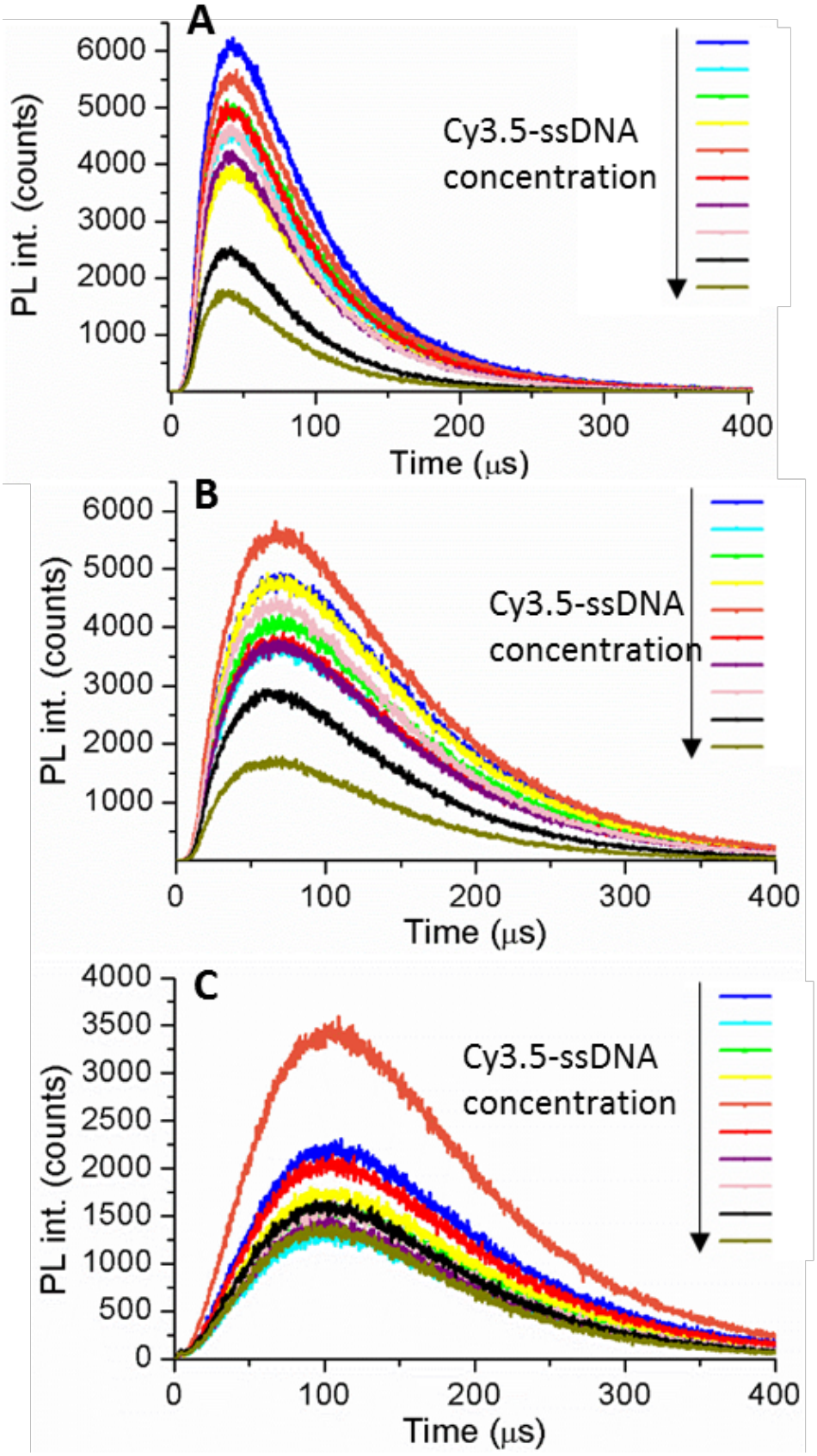

Figure S14- Kinetic profiles of UCNP-DNA nanohybrids in water (1 mg/mL) with different Cy3.5-ssDNA:UCNP ratios in the donor channel (λ_{em} = 541/20 nm) under 980 nm excitation for c-UCNPs (A) and css-UCNPs (B) and under 808 nm excitation for css-UCNPs (C).

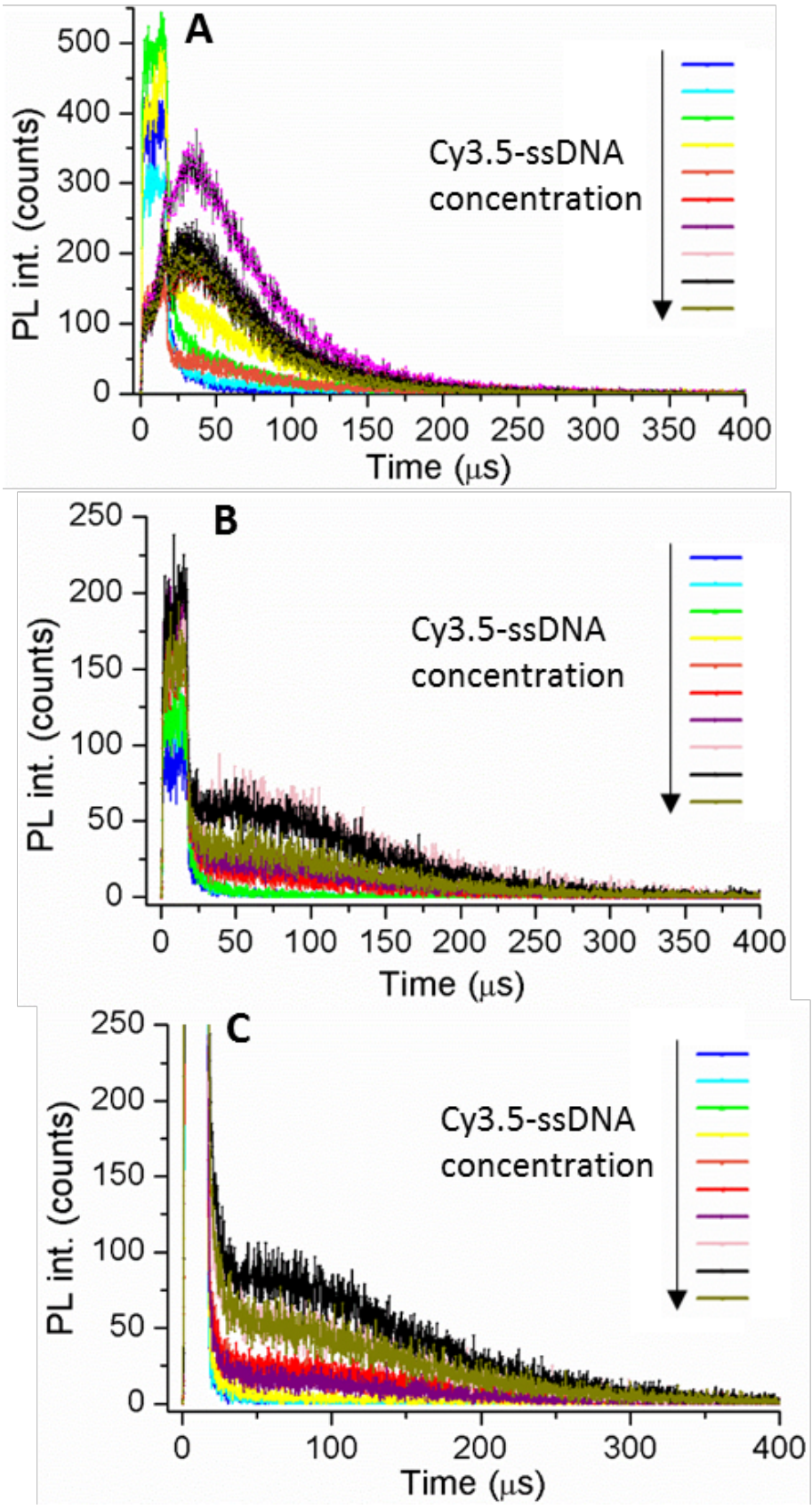

Figure S15- Kinetic profiles of UCNP-DNA nanohybrids in water (1 mg/mL) with different Cy3.5-ssDNA:UCNP ratios in the acceptor channel (λ_{em}=607/10 nm) under 980 nm excitation for c-UCNPs (A) and css-UCNPs (B) and under 808 nm excitation for css-UCNPs (C).

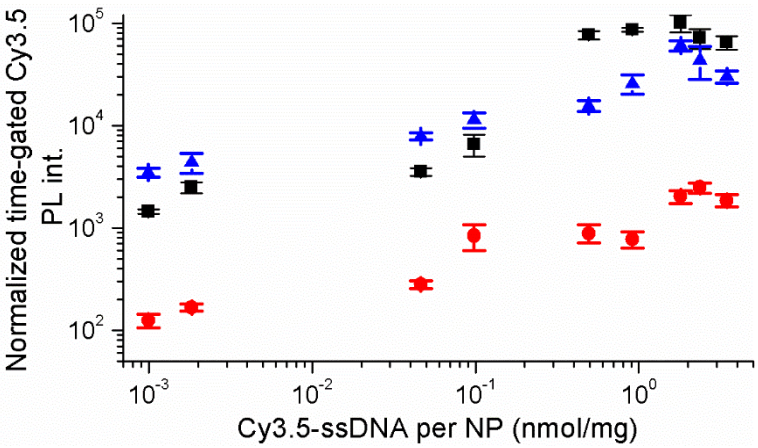

Figure S16- Time-gated PL intensity of Cy3.5 FRET-sensitized by c-UCNPs (black) and css-UCNPs (red) under 980 nm excitation and by css-UCNPs (blue) under 808 nm excitation. All values were calculated from the time-resolved intensities in Figure S15. First, all curves were normalized to the excitation power (whose differences can be seen in the first 20 µs of the curves in Figure S15). Then, the PL intensities were integrated from 20 µs (just after the excitation pulse) to 300 µs (when the decay has almost reached zero intensity).

REFERENCE

(1) Mackenzie, L. E.; Goode, J. A.; Vakurov, A.; Nampi, P. P.; Saha, S.; Jose, G.; Millner, P. A. The Theoretical Molecular Weight of NaYF4:RE Upconversion Nanoparticles. *Sci. Rep.* **2018**, *8* (1), 1106.