Supporting information for "Quantitative assessment of energy transfer in upconverting nanoparticles grafted with organic dyes"

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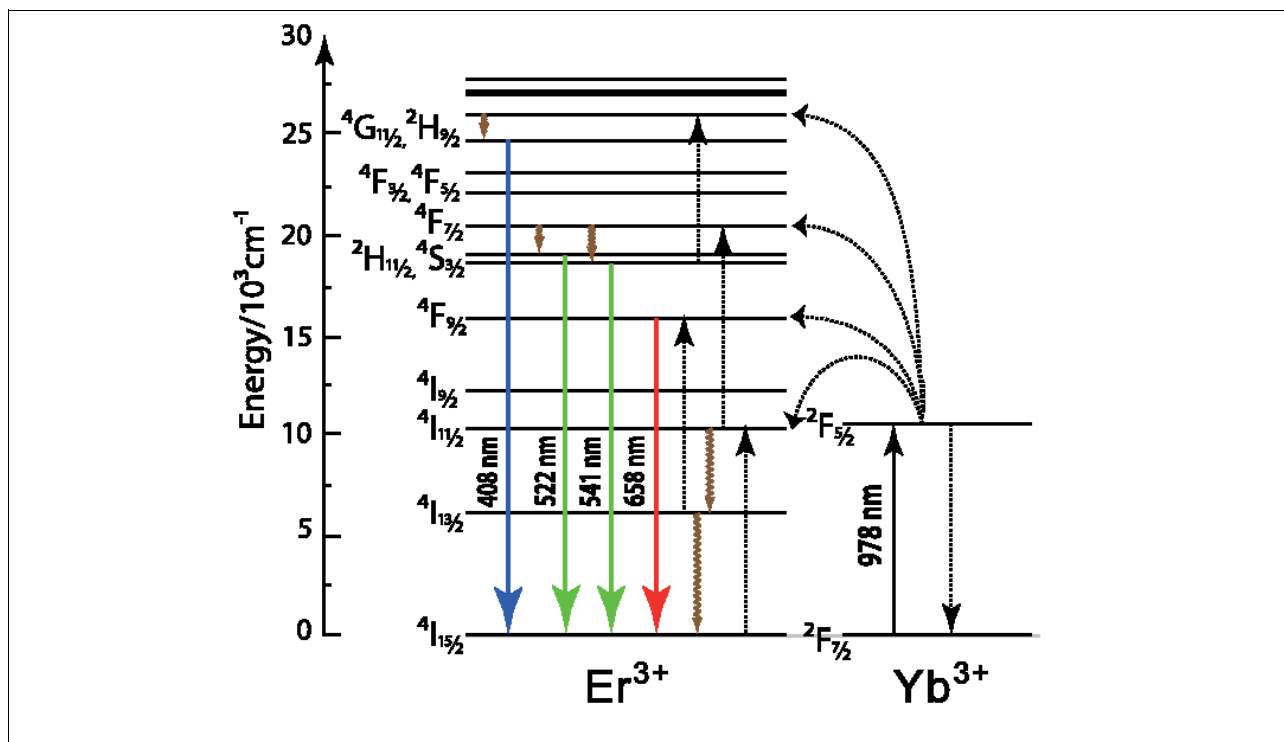9. References.

1. Scheme of UCNP upconversion mechanism

Fig. S1. Simplified upconversion mechanism in Yb-Er UCNPs. Reprinted from Haase and Schafer, 2015.[1]
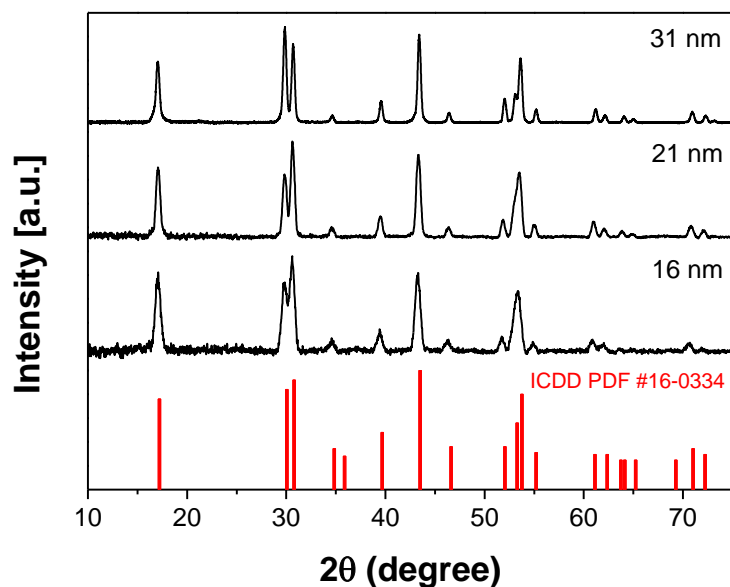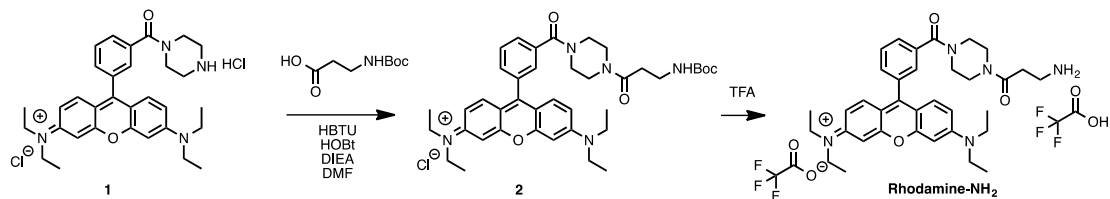
2. XRD data for raw UCNPs

Fig. S2: X-ray diffraction patterns of NaYF$_4$ (20% Yb, 2% Er, 0-20% Gd) nanocrystals with decreasing size from 31 nm to 16 nm (top to bottom) and the corresponding standard pattern of hexagonal phase NaYF$_4$ (red, ICDD PDF #16-0334).

3. Synthesis and spectra of Rhodamine-NH$_2$

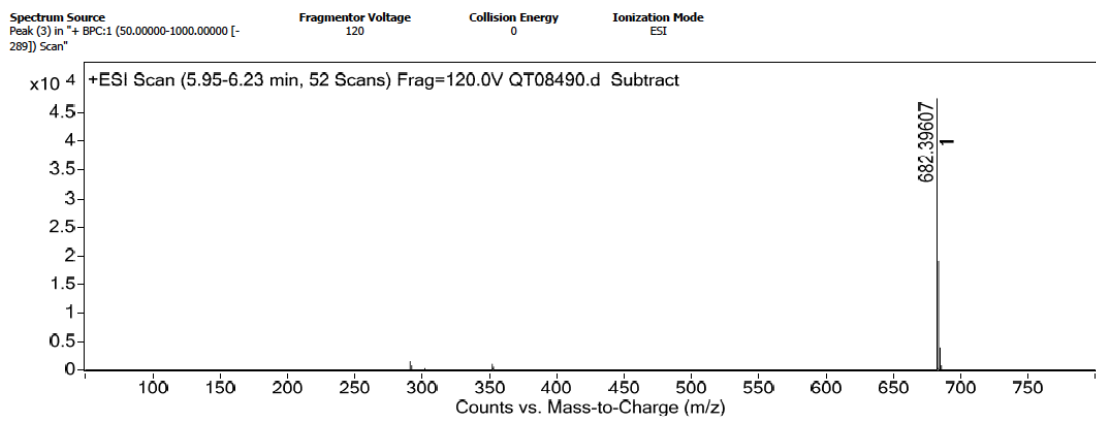*Fig. S3. Scheme of synthesis of Rhodamine-NH$_2$.*

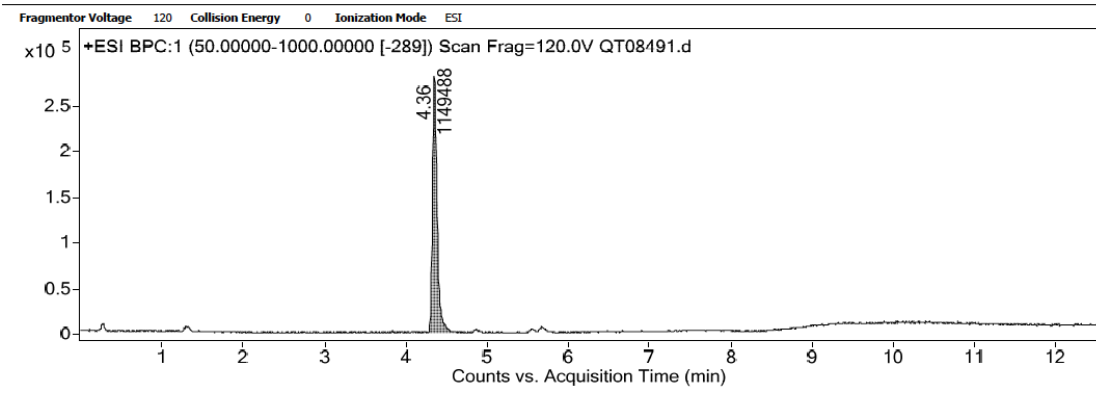**1** was synthesized according to a reported procedure. [2]

Synthesis of **2**. To a solution of **1** (210 mg, 0.360 mmol) in DMF (5 mL) was added N-boc-β-Alanine (72 mg, 0.360 mmol, 1 eq), HOBt (58 mg, 0.360 mmol, 1 eq) and HBTU (145 mg, 0.360 mmol, 1 eq). The solution was stirred under argon and DIEA (188 μL, 1.080 mmol, 3 eq) was added. The mixture was allowed to stir overnight under argon. The solvents were evaporated and the product was extracted with DCM, then washed with water before being dried over MgSO$_4$. The solution was filtered and evaporated. The crude was purified by column chromatography on silica gel (DCM/MeOH : 94/6) to give 250 mg of **2** as dark pink foam (yield= 96%). Rf=0.42 (DCM/MeOH : 96/4). ^{1}H-NMR (400 MHz, CDCl$_3$): 7.60 (m, 2H, H Ar), 7.46 (m, 1H, H Ar),7.24-7.13 (m, 3H, H Ar), 6.95-6.80 (m, 2H, H Ar), 6.72-6.66 (m, 2H, H Ar), 5.20 (m, 1H, NH), 3.59-3.49 (m, 8H, 4 CH$_2$),3.40-3.25 (m, 10H, 5 CH$_2$), 2.46 (m, 2H, CH$_2$), 1.34-1.21 (m, 21H, CH$_3$ Boc, 4 CH$_3$). ^{13}C-NMR (100 MHz, CDCl$_3$): 170.7 (CO), 170.3 (CO), 167.7 (CO), 162.5 (C Ar), 157.6 (C Ar), 155.8 (C Ar), 155.6 (C Ar), 135.0 (C Ar), 131.9 (C Ar), 130.9 (C Ar), 130.2 (C Ar), 130.0 (C Ar), 127.4 (C Ar), 114.3 (C Ar), 114.0 (C Ar), 113.7 (C Ar), 96.4, 46.0 (CH$_2$ Et), 45.1-44.8 (multiple peaks), 41.6-40.8 (multiple peaks), 38.5, 36.4-36.2, 33.3, 31.3, 28.4 (CH$_3$ Boc), 12.5 (CH$_3$ Et). HRMS (ES$^+$), calcd for C$_{40}$H$_{52}$N$_5$O$_5$ [M]$^+$ 682.3963, found 682.3960.

Synthesis of **Rhodamine-NH$_2$**. To a solution of **2** (250 mg, 0.348 mmol) in DCM (5 mL) was added TFA (4 mL), the solution was sonicated for 20 s and the solvents were evaporated. The crude was then dissolved in a minimum of DCM and the solution was poured in stirring Et$_2$O. The solution was filtered to obtain 195 mg of Rhodamine-NH$_2$ as a deep red solid (yield = 69%). Rf=0.15 (DCM/MeOH : 95/5). The purity was checked by ^{1}H-NMR and LC-HRMS. ^{1}H-NMR (400 MHz, DMSO-d6): 7.76-7.72 (m, 6H, H Ar, NH$_3$$^+$), 7.54 (s, 1H, H Ar), 7.18-7.10 (m, 4H, H Ar), 6.96 (s, 2H, H Ar), 3.66 (q, 8H, CH$_2$ Et), 3.39 (m, 12H, CH$_2$ pip, CH$_2$ β-ala), 1,24 (t, 12H, CH$_3$ Et). HRMS (ES$^+$), calcd for C$_{35}$H$_{44}$N$_5$O$_3$ [M+H]$^+$ 582.3439, found 582.3434.

Fig. S4. ^1H NMR spectrum of 2 (CDCl$_3$)

Fig. S5. ^{13}C NMR spectrum of 2 (CDCl$_3$)
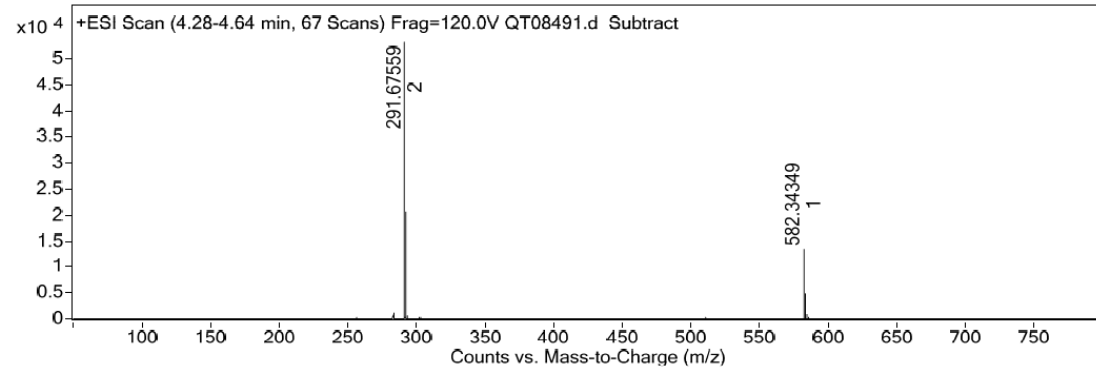
*Fig. S6. HRMS spectrum of **2***

*Fig. S7. Chromatogram of **Rhodamine-NH$_2$** (ACN 0.1% formic acid / Water 0.1% formic acid ; C-18 column)*

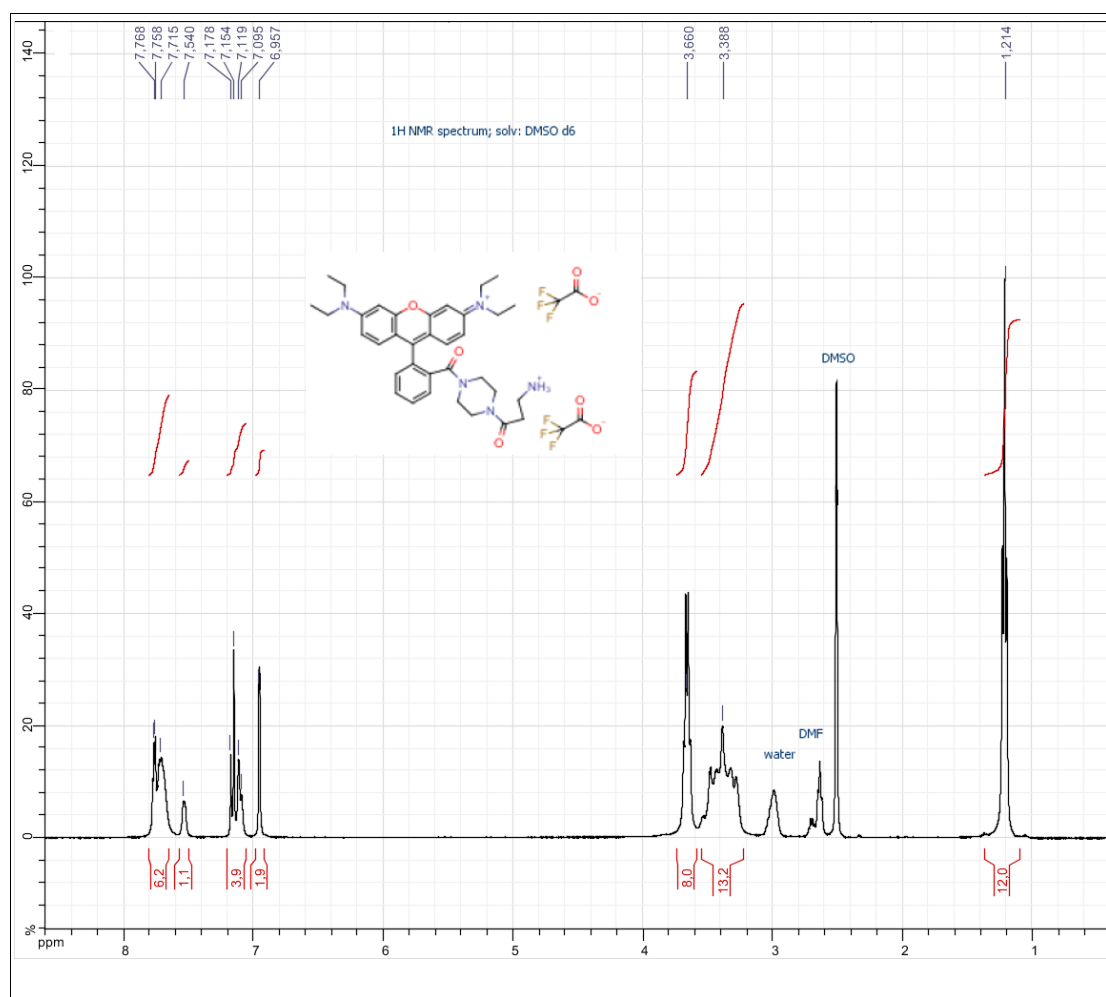*Fig. S8. HRMS spectrum of **Rhodamine-NH$_2$***

Fig. S9. ^{1}H NMR spectrum of Rhodamine-NH$_2$ (DMSO-d6)

4. Synthesis of the PMA amphiphilic polymer

In a 10 mL flask with a septum and flushed with argon, 18 mg dodecylamine (97 µmol, 0.75eq) were dissolved in 2 mL anhydrous DMF under magnetic stirring. Diisopropylethylamine (67 µL, 390 µmol, 3eq) was added. 1 mg of piperidyl-beta-alanine-coupled Rhodamine B (1.3 µmol, 0.01 eq) was added. The solution was allowed to stir for 10 min, then 20 mg of poly(isobutylene-alt-maleic anhydride) were added in one portion (130 µmol, 1 eq monomer, avg MW 6000). The vessel was purged with argon a second time, and the reaction mass was stirred at room temperature. After 30 min, a drop of water (approx. 100 eq) was added. The reaction mass was evaporated, redissolved in dichloromethane and purified on LH20 size-exclusion chromatography column (eluent: dichloromethane-methanol 1:1 v/v). Combined elutes were evaporated, and the residue was redissolved in 2.6 mL spectral grade chloroform, corresponding to a theoretical 0.05 M concentration (assuming quantitative yield). This solution was used as a stock for subsequent coating of UCNPs.

5. Polymer coating of UCNPs and calculation of dye quantity per particle

We used a modified version of the protocol from Wilhelm et al., 2015.[3] 0.33 mL of UCNPs with 20.6 nm diameter (5 mg/mL) in cyclohexane were mixed with 0.42 mL 0.05 M PMA solution, sonicated for 1 min at room temperature, and evaporated. The residue was dissolved in 0.75 mL spectral grade chloroform and sonicated for 1 min at room temperature. Then, 1 mL 0.01 M NaOH were added. The mixture was vortexed for 30 s and slowly evaporated on rotavap (high speed of rotation and low vacuum are recommended to avoid bumping), until only aqueous phase remained. Obtained phase was filtered through a Millex GP syringe filter (0.22 µm pore size). The filter was

washed once with 1 mL 0.01 M NaOH. Obtained filtrates were combined.

The quantity of polymer stock solution required for the coating process was calculated using:

$$V_{\text{polymersolution}} = R_P \pi \frac{\omega_{\text{UCNP}}}{\rho V_{\text{UCNP}}} d_{\text{eff}}^2 \frac{1}{c_{\text{polymer}}}$$

where R_p is the number of polymer, expressed in monomers, applied per nm^2 of UCNP surface (100 in case of UCNP coated with oleic acid, which is about 5 times in excess compared to the tight fatty chain packing on surface), ω_{UCNP} is the mass concentration of UCNPs (e.g. mg/mL), ρ is the density of UCNPs ($4.21 \cdot 10^{-21}$ g/nm^3), V is the volume of UCNP in nm^3, d_{eff} is the effective diameter of UCNP, which includes the thickness of the oleic acid layer (e.g. for a 20.6 nm diameter the effective diameter is 21.7 nm, because of two 0.55 nm thick layers of oleic acid).

The number of surface dyes per particle can be calculated by:

$$N = \pi(d+2l)^2 * C_{f.ch.} * P$$

where d is the diameter of the particle (nm), l is the thickness of the polymer-oleic acid layer (nm), $C_{f.ch.}$ is the number of fatty chains per nm^2 in the tightly packed monolayer, and P is the percentage of dyes per monomer.
The calculated number of dyes per particle is provided in following table (thickness of oleic acid layer assumed to be 1.1 nm, packing density of fatty chains in monolayer is assumed to be 5 nm^{-2}). It should be noted that this calculation represents the quantity of dyes bound specifically on the surface of UCNPs. Due to excess of polymer being used to ensure proper UCNP coating, some polymer micelles are formed in dispersion. Dyes in polymer micelles are not considered surface-bound to UCNPs and are not accounted for in the calculation.

particle diameter, nm	*dye percentage per monomer* quantity of surface-bound dyes per particle after coating process			
	0%	**0.33%**	**1.5%**	**6.6%**
16	0	17	78	343
21	0	27	123	539
31	0	54	244	1075

particle diameter, nm	*dye:Er^{3+} ratio*			
	0%	**0.33%**	**1.5%**	**6.6%**
16	0	0.043	0.196	0.861
21	0	0.035	0.157	0.692
31	0	0.020	0.093	0.409

Table S1. Calculated dye quantities and dye-Er^{3+} ratios per particle.
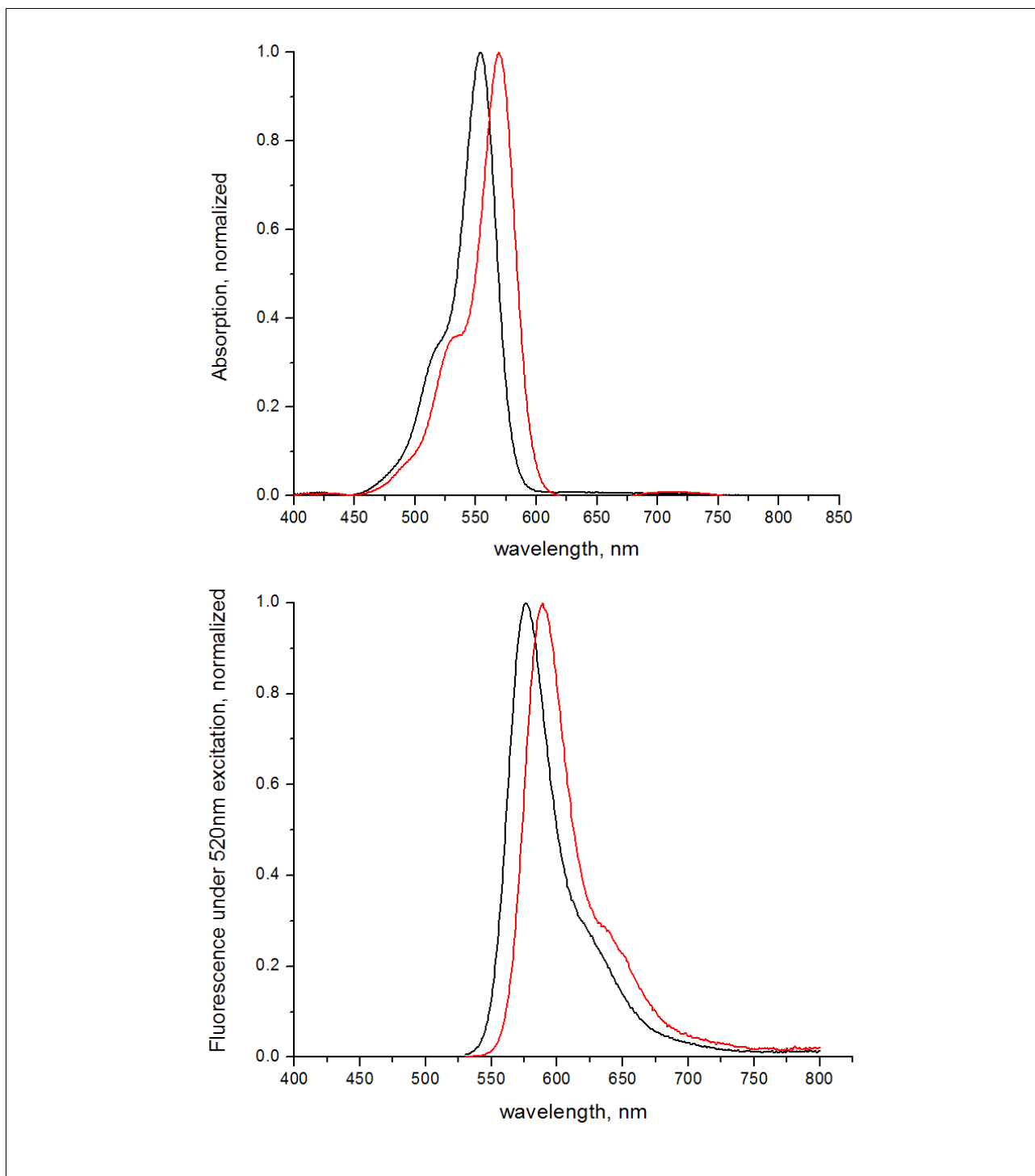
Fig. S10. Normalized absorption and emission spectra of Rhodamine B (black) and UCNP-PMA-RhB conjugates in water (red). Absorption spectra were measured on a Cary 4000 spectrometer. Fluorescence spectra were recorded with a 520 nm excitation wavelength on a Fluoromax-4 spectrofluorimeter.

6. Characterization of the UCNP-PMA-RhB by Dynamic Light Scattering (DLS)

Size distributions have been obtained by "volume" treatment of the correlation curves. This type of treatment was chosen due to overestimation of the percentage of larger particles by "intensity" treatment. All curves and polydispersity indexes (PdI) were calculated as an average of three consecutive measurements. Measurements were done at 298 K. Each curve in figures is an average of 3 measurements of 3 samples of one type. PdI values are given as a mean ± 1 standard deviation. Samples were diluted 10× in mQ water.
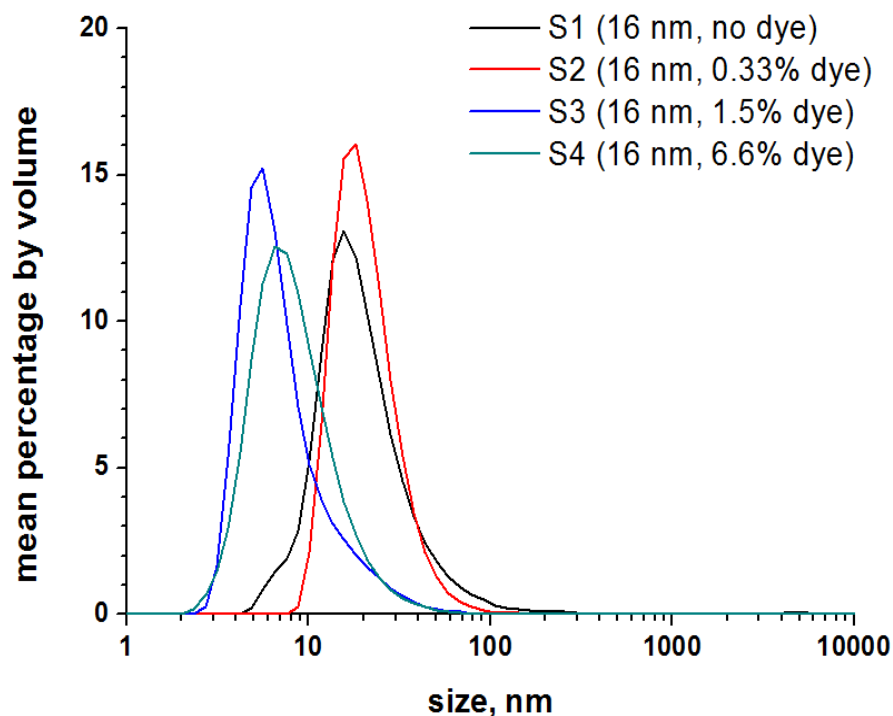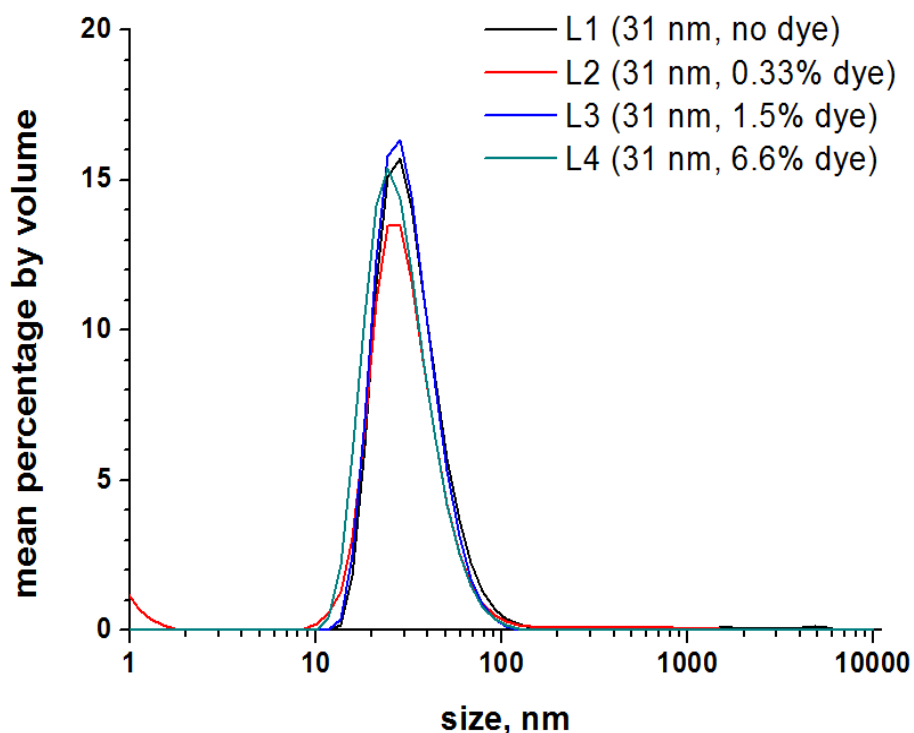
*Fig. S11. Mean size distributions for samples S1-S4. PdI are 0.369±0.047, 0.341±0.020, 0.428±0.046, and 0.432±0.033, respectively.*
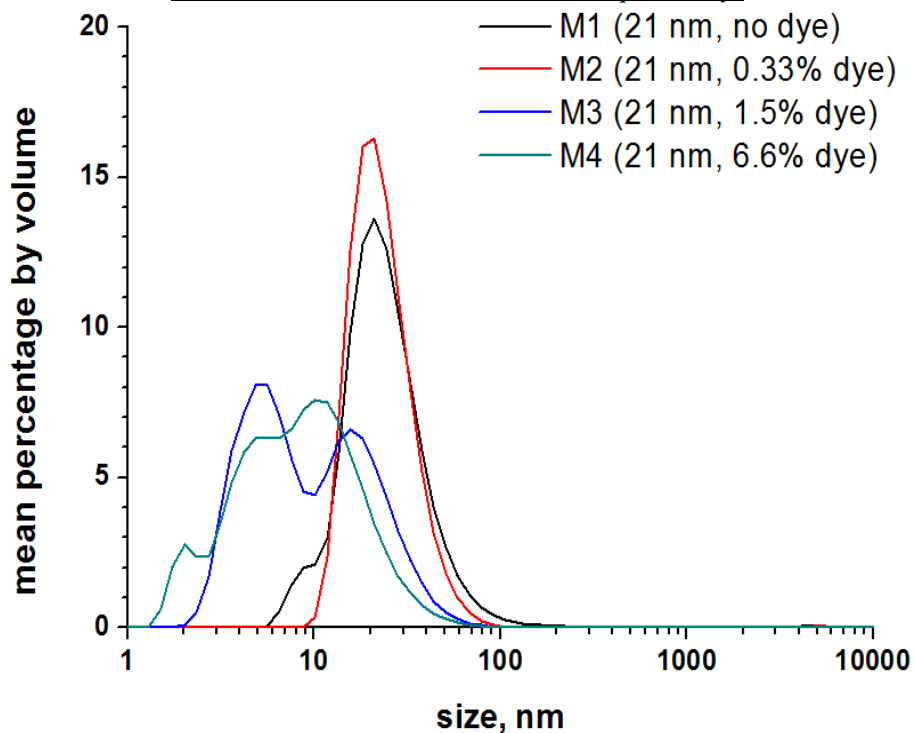
*Fig. S12. Mean size distributions for samples M1-M4. PdI are 0.296±0.037, 0.191±0.021, 0.260±0.048, and 0.302±0.034, respectively.*

Fig. S13. Mean size distributions for samples L1-L4. PdI are 0.230±0.038, 0.199±0.088, 0.149±0.027, and 0.186±0.032, respectively.

7. Emission spectra of the dye-grafted UCNPs

All spectra were measured in samples diluted 10× in milliQ water, with continuous 980 nm excitation. The position of the beam inside the cuvette was chosen to be as close to the detector as possible, to mitigate any possible inner filter effects (see Figure S20 for details). Each spectrum represents an average of 480 spectra, each with signal accumulation of 5 s. After averaging, the spectra are smoothed (adjacent averaging, 5 points, corresponds to ~1nm spectral resolution), normalized by the intensity at 664 nm (to minimize dye crosstalk), and the bands are integrated to obtain the intensities used in equation 1 (510-565 nm for the green band and 655-680 nm for the red band).
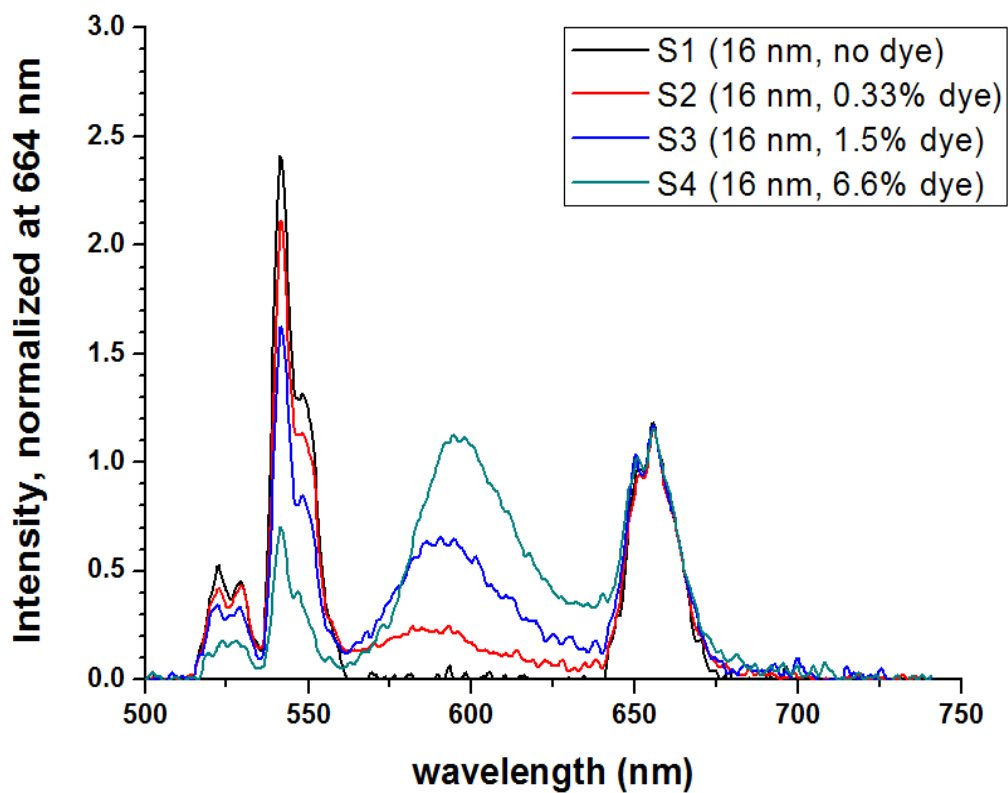
Fig. S14. Mean normalized luminescence spectra of samples S1-S4. Excitation wavelength 980 nm.
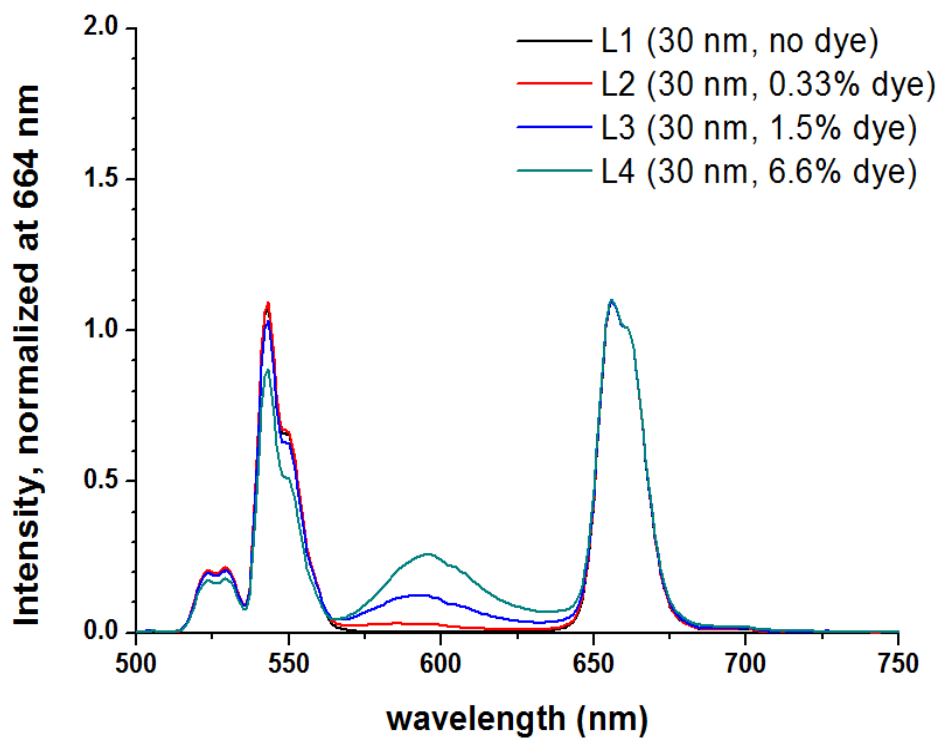

Fig. S15. Mean normalized luminescence spectra of samples L1-L4. Excitation wavelength 980 nm.

## 8. Emission decays of the dye-grafted UCNPs

All decays were collected to obtain total 2M photons per decay curve (including the truncated rising part). For each sample 5 curves have been collected and independently fitted. Values provided in the table are mean ± 1 standard deviation over 15 decays in total (5 curves x 3 sample repeats).
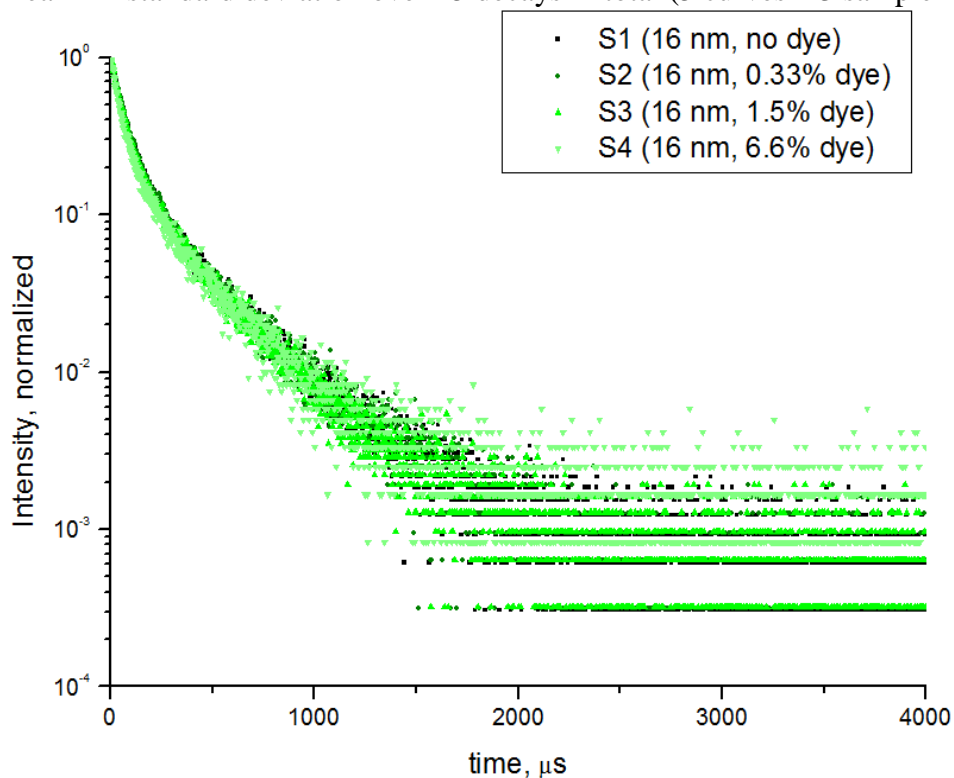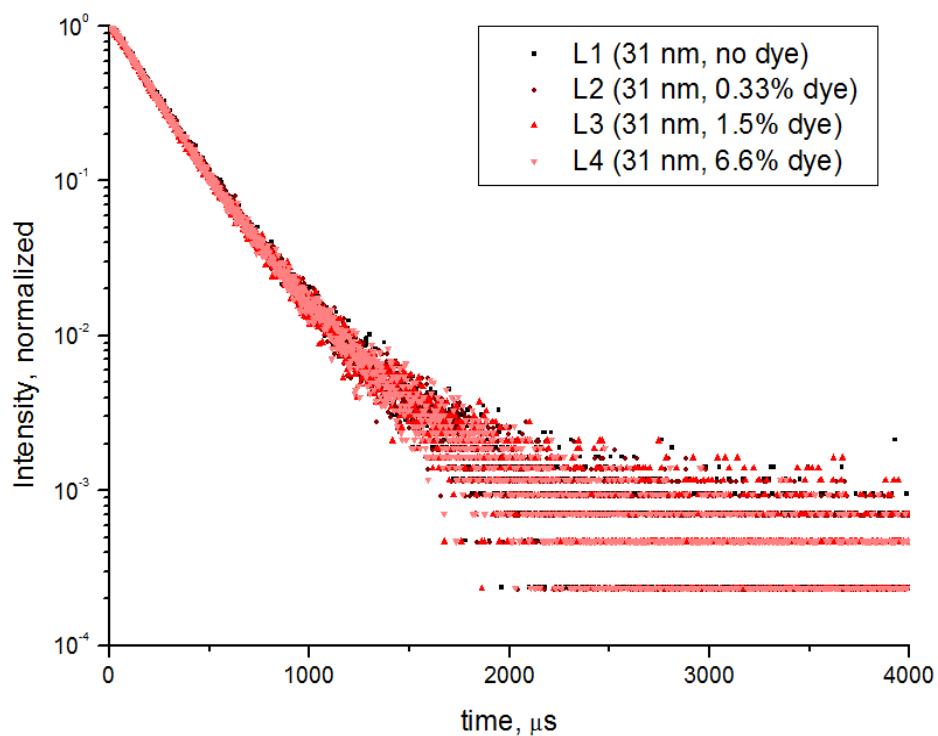

Fig. S16. Examples of normalized decay curves of one batch of samples S1-S4 at 542 nm emission. Excitation wavelength 980 nm.
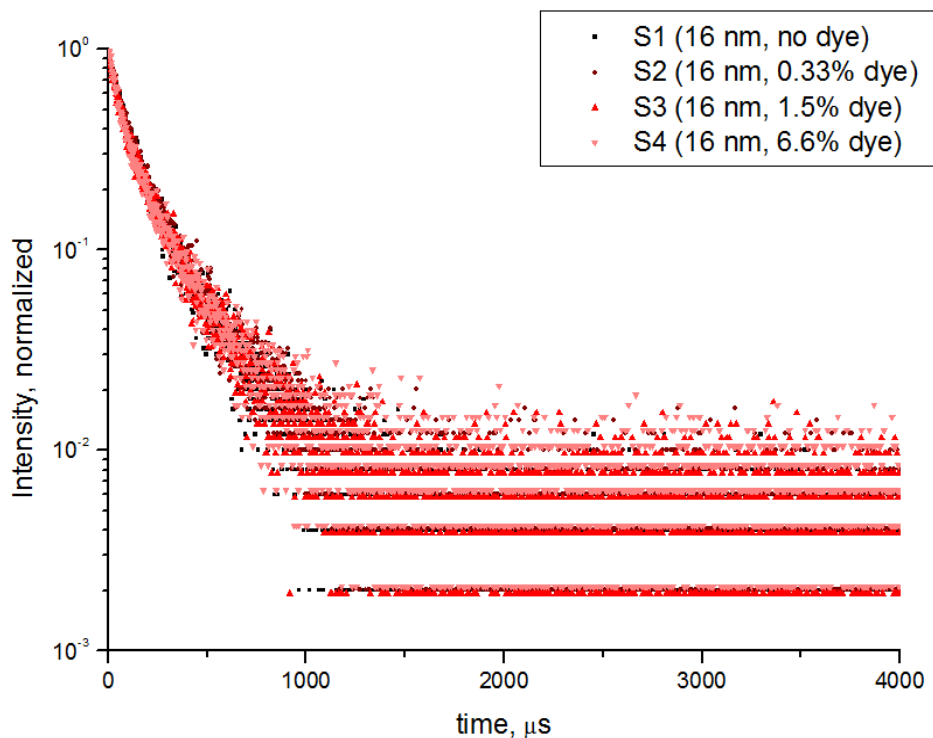
Fig. S17. Examples of normalized decay curves of one batch of samples S1-S4 at 542 nm emission. Excitation wavelength 980 nm.
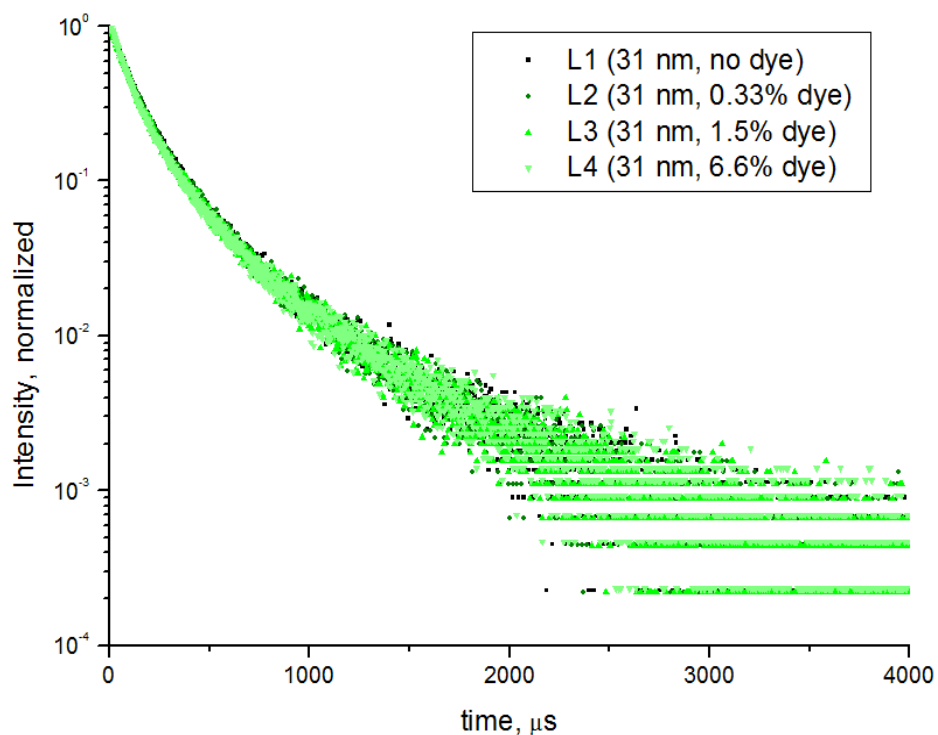
Fig. S18. Examples of normalized decay curves of one batch of samples L1-L4 at 542 nm emission. Excitation wavelength 980 nm.

Fig. S19. Examples of normalized decay curves of one batch of samples L1-L4 at 662 nm emission. Excitation wavelength 980 nm.

16 nm diameter

	S1, 542nm	S2, 542nm	S3, 542nm	S4, 542nm
A1	0.68 ± 0.01	0.62 ± 0.08	0.65 ± 0.06	0.68 ± 0.05
t1	60 ± 6	51 ± 6	49 ± 5	42 ± 3
A2	0.20 ± 0.02	0.25 ± 0.06	0.22 ± 0.05	0.20 ± 0.04
t2	149 ± 8	122 ± 22	130 ± 29	134 ± 41
A3	0.12 ± 0.01	0.14 ± 0.03	0.13 ± 0.02	0.12 ± 0.04
t3	364 ± 35	331 ± 26	329 ± 30	348 ± 69

	S1, 662nm	S2, 662nm	S3, 662nm	S4, 662nm
A1	0.57 ± 0.10	0.53 ± 0.04	0.53 ± 0.05	0.57 ± 0.06
t1	76 ± 14	65 ± 9	66 ± 11	53 ± 9
A2	0.43 ± 0.10	0.47 ± 0.04	0.47 ± 0.05	0.43 ± 0.06
t2	236 ± 13	223 ± 15	228 ± 14	224 ± 11

spectral ratio	2.06 ± 0.49	1.88 ± 0.21	1.37 ± 0.15	0.58 ± 0.12

21 nm diameter

	M1, 542nm	M2, 542nm	M3, 542nm	M4, 542nm
A1	0.51 ± 0.08	0.55 ± 0.06	0.49 ± 0.06	0.59 ± 0.03
t1	58 ± 6	58 ± 4	50 ± 4	46 ± 2
A2	0.31 ± 0.08	0.26 ± 0.06	0.31 ± 0.06	0.24 ± 0.03
t2	127 ± 13	131 ± 12	114 ± 10	123 ± 7
A3	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01
t3	345 ± 6	329 ± 9	321 ± 6	332 ± 11

	M1, 662nm	M2, 662nm	M3, 662nm	M4, 662nm
A1	0.87 ± 0.02	0.86 ± 0.03	0.85 ± 0.03	0.88 ± 0.02
t1	108 ± 4	103 ± 8	95 ± 7	92 ± 8
A2	0.13 ± 0.02	0.14 ± 0.03	0.15 ± 0.03	0.12 ± 0.03
t2	302 ± 16	297 ± 24	278 ± 23	297 ± 24

spectral ratio	2.35 ± 0.16	2.04 ± 0.63	1.95 ± 0.57	1.49 ± 0.15

30 nm diameter

	L1, 542nm	L2, 542nm	L3, 542nm	L4, 542nm
A1	0.56 ± 0.03	0.56 ± 0.03	0.58 ± 0.03	0.61 ± 0.03
t1	86 ± 4	85 ± 4	83 ± 3	77 ± 3
A2	0.37 ± 0.03	0.37 ± 0.02	0.35 ± 0.03	0.33 ± 0.02
t2	195 ± 9	192 ± 11	193 ± 10	195 ± 10
A3	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
t3	551 ± 19	543 ± 26	539 ± 21	549 ± 15

	L1, 662nm	L2, 662nm	L3, 662nm	L4, 662nm
A1	0.91 ± 0.01	0.91 ± 0.01	0.88 ± 0.02	0.87 ± 0.02
t1	198 ± 5	196 ± 9	191 ± 5	188 ± 6
A2	0.09 ± 0.01	0.09 ± 0.01	0.12 ± 0.02	0.13 ± 0.02
t2	398 ± 19	396 ± 23	371 ± 14	369 ± 16

spectral ratio	1.10 ± 0.03	1.12 ± 0.07	1.09 ± 0.03	0.88 ± 0.07

Table S2. Bi- and triexponential decay fit parameters for all samples. Decay lifetimes are given in µs. Preexponential parameters are unitless. All values are given as a mean ± 1 standard deviation. The choice of the number of components was determined by maximum entropy method. All samples were measured diluted 10x in milliQ water, with 8 kW/cm2 continuous-wave 980 nm excitation.
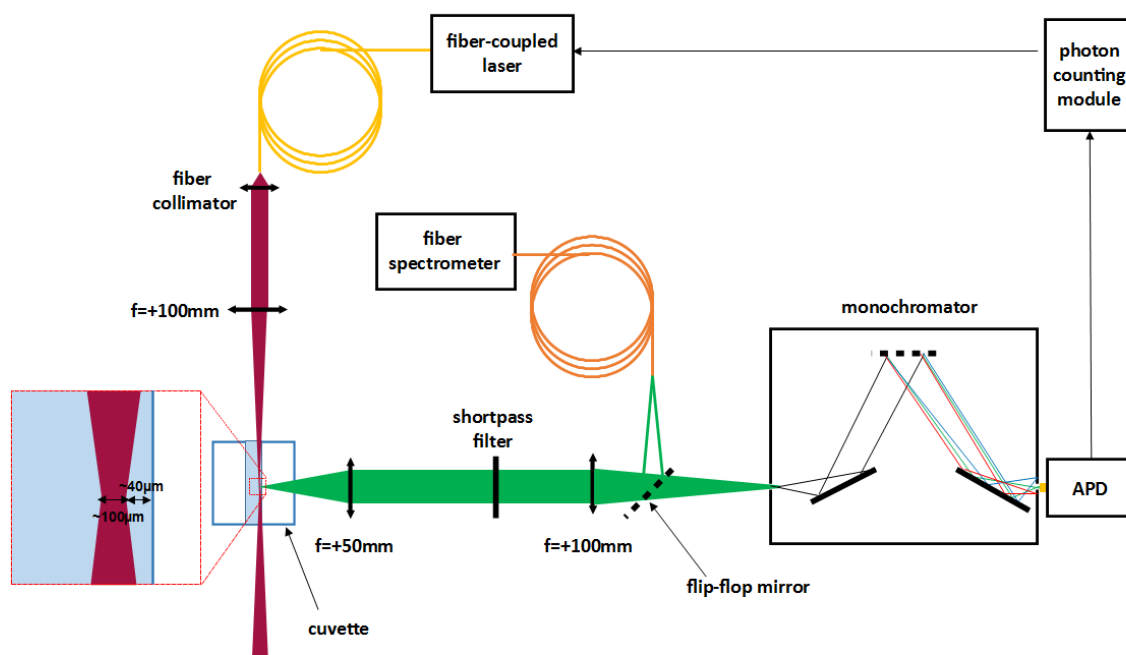
Fig. S20. Setup scheme.

9. References.

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(2) Nguyen, T.; Francis, M. B. Practical Synthetic Route to Functionalized Rhodamine Dyes. *Org. Lett.* **2003**, *5*, 3245–3248.

(3) Wilhelm, S.; Kaiser, M.; Würth, C.; Heiland, J.; Carrillo-Carrion, C.; Muhr, V.; Wolfbeis, O. S.; Parak, W. J.; Resch-Genger, U.; Hirsch, T. Water Dispersible Upconverting Nanoparticles: Effects of Surface Modification on Their Luminescence and Colloidal Stability. *Nanoscale* **2015**, *7*, 1403–1410.