Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting information

Aluminium-based fluorinated counterion for enhanced encapsulation and emission of dyes in biodegradable polymer nanoparticles

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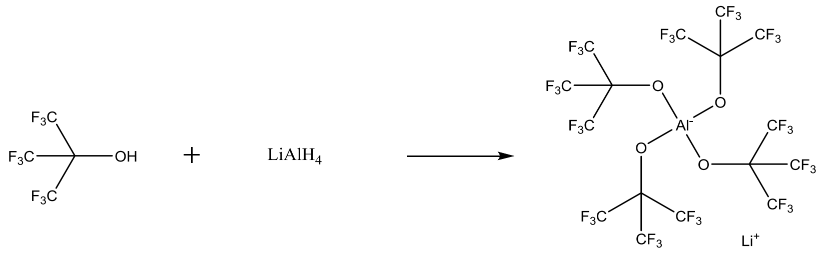
Chemical synthesis

R18/F5-TPB

Synthesis of Rhodamine B octadecyl ester tetrakis(pentafluorophenyl)borate (**R18/F5-TPB**) was described previously.[1]

Li[F9-Al]

Compound was prepared according to a modified protocol.[2]



27%
Li[Al-F9]

An oven-dried 250 ml 3-necked flask with a stirring bar was vacuumated and argonated several times, afterwards 1M $LiAlH_4$ solution in ether (1 eq., 20 mL, 20 mmol) was added through a syringe and the flask was put on a rotavap to evaporate ether. After the liquid was evaporated, temperature of the bath was increased to 80 ^oC and LAH was left do dry for 2,5 hours more at this temperature. After drying and cooling, the flask was argonated and put to an oil bath on the stirring plate; a reflux condenser and a bubbler outlet were attached. The system was vacuumated and argonated 3 times, and dry toluene (70 mL) was added through a syringe. 3 ml of perfluoro-tert-butanol were added while stirring, but no gas evolution was observed, and reaction mixture was heated to 80^{o}C, when the strong gas evolution started and reaction mixture began to warm itself, so it was taken out of the bath until the reaction mixture calmed down. Afterwards, the flask was put back into an oil bath, and the rest of perfluoro-tert-butanol (11 ml, 5eq in total) was added dropwise.

Reaction mixture was left for 2 hrs at 80 °C and then temperature was risen to 100°C for one hour, afterwards the reaction was left at 50 °C over the weekend.

Afterwards, the reaction mixture was heated up to reflux and hot filtration was performed, separating greyish insoluble powder from toluene solution. Solution was let to cool down, put to freezer, and precipitated white crystals were filtered and washed with cold toluene and dried under vacuum, which yielded 5.23 g (5.37 mmol, 27 %) of **Li[F9-Al]**.

Decreased product yields and weaker reactivity of LAH comparing to original protocol could be explained by insufficiently dry toluene and/or argon.

^{19}F NMR (376 MHz, DMSO-d_6) δ ppm -75.10 (s)

MS (m/z): [M]- calcd. for $C_{16}AlF_{36}O_4$, 966.904; found 966.815

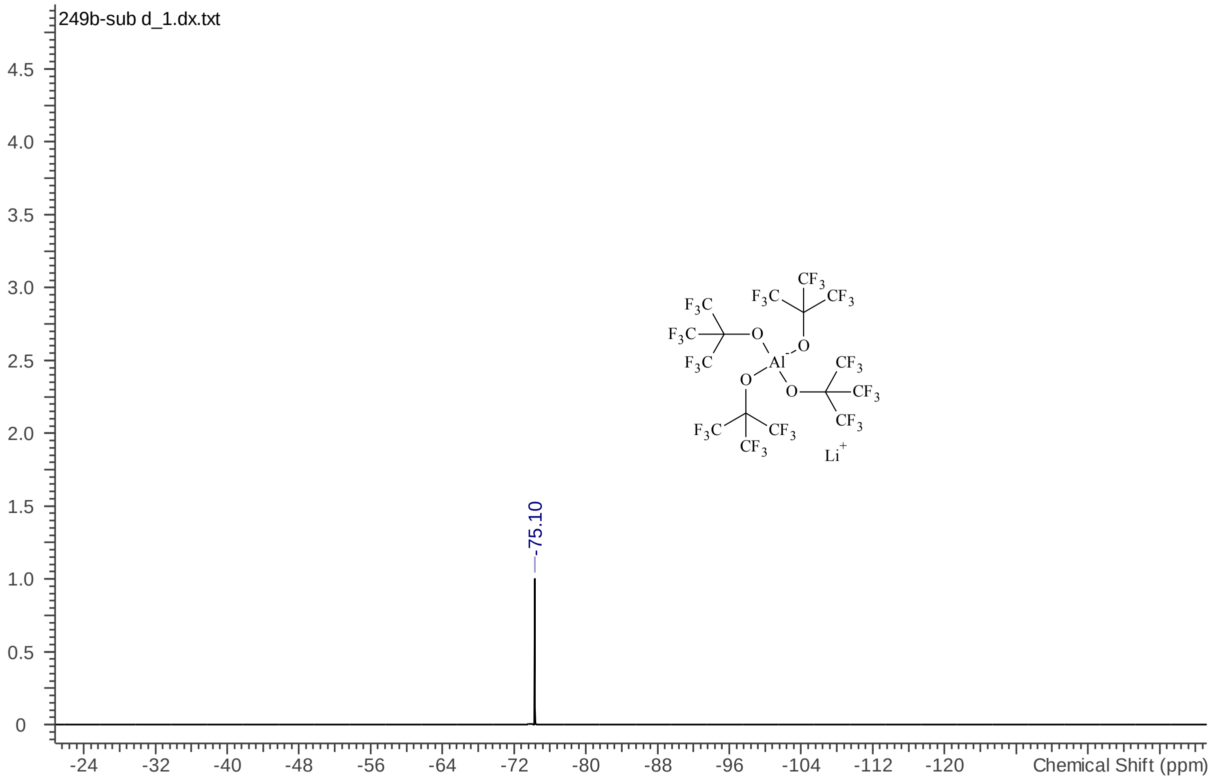
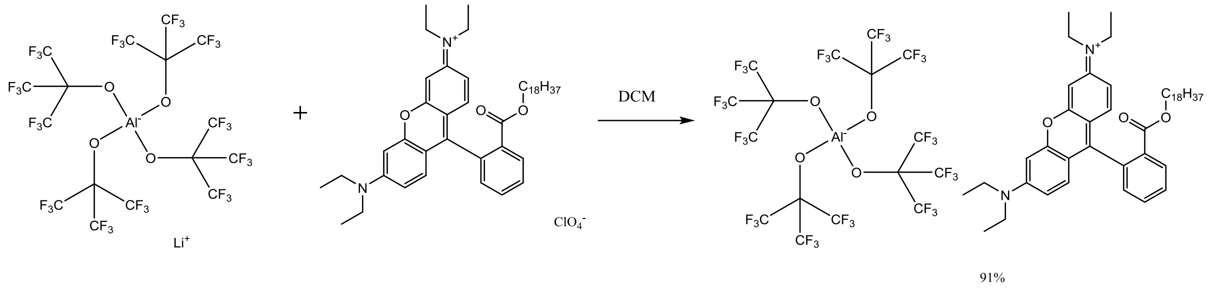


Figure S1. ^{19}F NMR of **Li[F9-Al]** in DMSO-d6.

R18/F9-Al



R18-Al-F9

Rhodamine B octadecyl ester perchlorate (1 eq., 8 mg, 0.0101 mmol) and Li[F9-Al] (3 eq., 29.4 mg, 0.0302 mmol) were mixed in DCM and sonicated for 20 sec, TLC (MeOH/DCM 5/95) has shown full conversion. Product was purified by column chromatography on silica, using DCM/MeOH 98/2 as eluent. After evaporation of solvents purple viscous oil of **R18/F9-Al** was obtained (15.2 mg, 0.00915 mmol, 91 %)

^{1}H NMR (500 MHz, ACETONITRILE-d_3) δ ppm 8.30 (1 H, d, *J*=7.7 Hz), 7.80 - 7.87 (2 H, m), 7.40 (1 H, d, *J*=7.4 Hz), 7.11 (2 H, d, *J*=9.5 Hz), 6.97 (2 H, dd, *J*=9.6, 2.6 Hz), 6.86 (2 H, d, *J*=2.4 Hz), 3.94 (2 H, t, *J*=6.4 Hz), 3.64 (8 H, q, *J*=7.3 Hz), 1.23 - 1.30 (40 H, m), 1.09 - 1.16 (4 H, m), 0.94 - 0.97 (2 H, m), 0.89 - 0.92 (3 H, m)

^{13}C NMR (126 MHz, ACETONITRILE-d_3) δ ppm 166.07 (s), 163.53 (s), 159.30 (s), 158.42 (s), 156.28 (s), 133.73 (s), 133.34 (s), 131.87 (s), 131.66 (s), 131.21 (s), 130.87 (s), 130.75 (s), 121.78 (q, *J*=288Hz), 114.90 (s), 114.08 (s), 96.49 (s), 66.05 (s), 46.25 (s), 32.25 (s), 29.99 (s), 29.98 (s), 29.97 (s), 29.96 (s), 29.89 (s), 29.87 (s), 29.68 (s), 29.68 (s), 29.47 (s), 28.60 (s), 26.13 (s), 23.00 (s), 13.99 (s), 12.39 (s)

^{19}F NMR (471 MHz, ACETONITRILE-d_3) δ ppm -75.98 (s)

HRMS (m/z): [M]+ calcd. for $C_{46}H_{67}N_2O_3^+$, 695.51517; found 695.51376
[M]- calcd. for $C_{16}AlF_{36}O_4$, 966.90371; found 966.89781

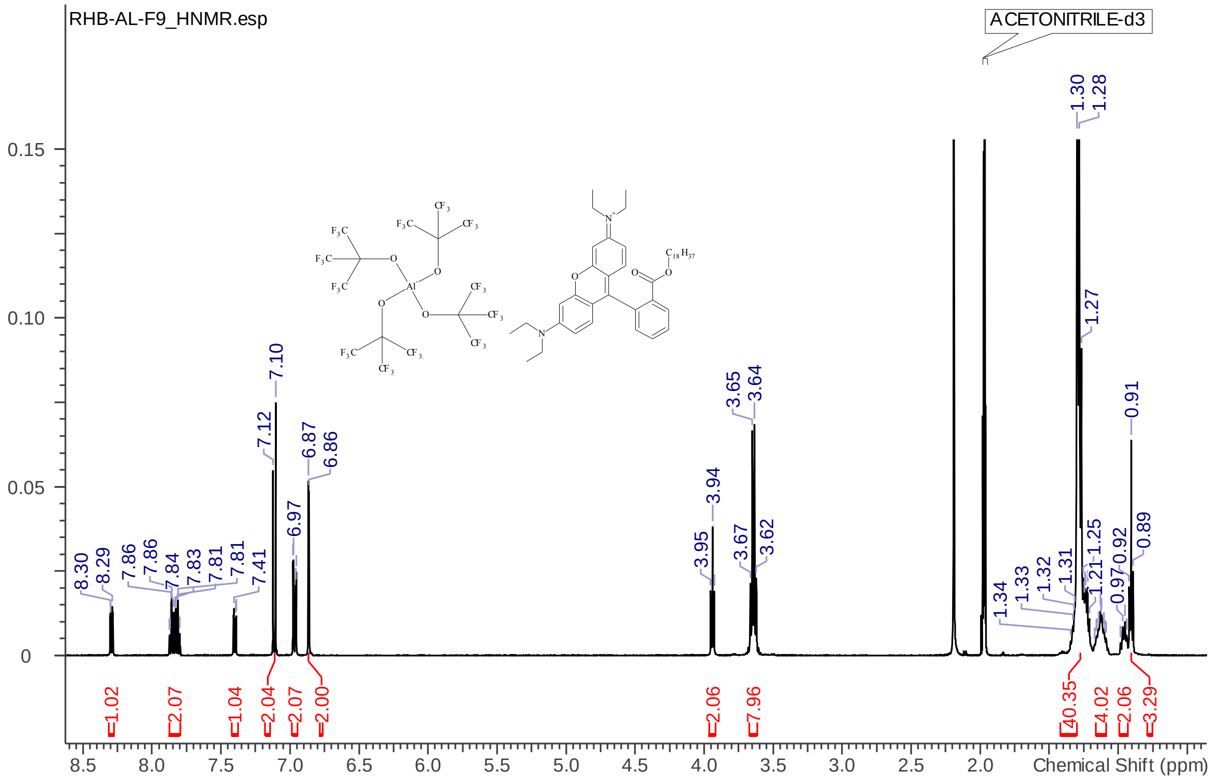


Figure S2. ^{1}H NMR of **R18/F9-Al** in MeCN-d3.

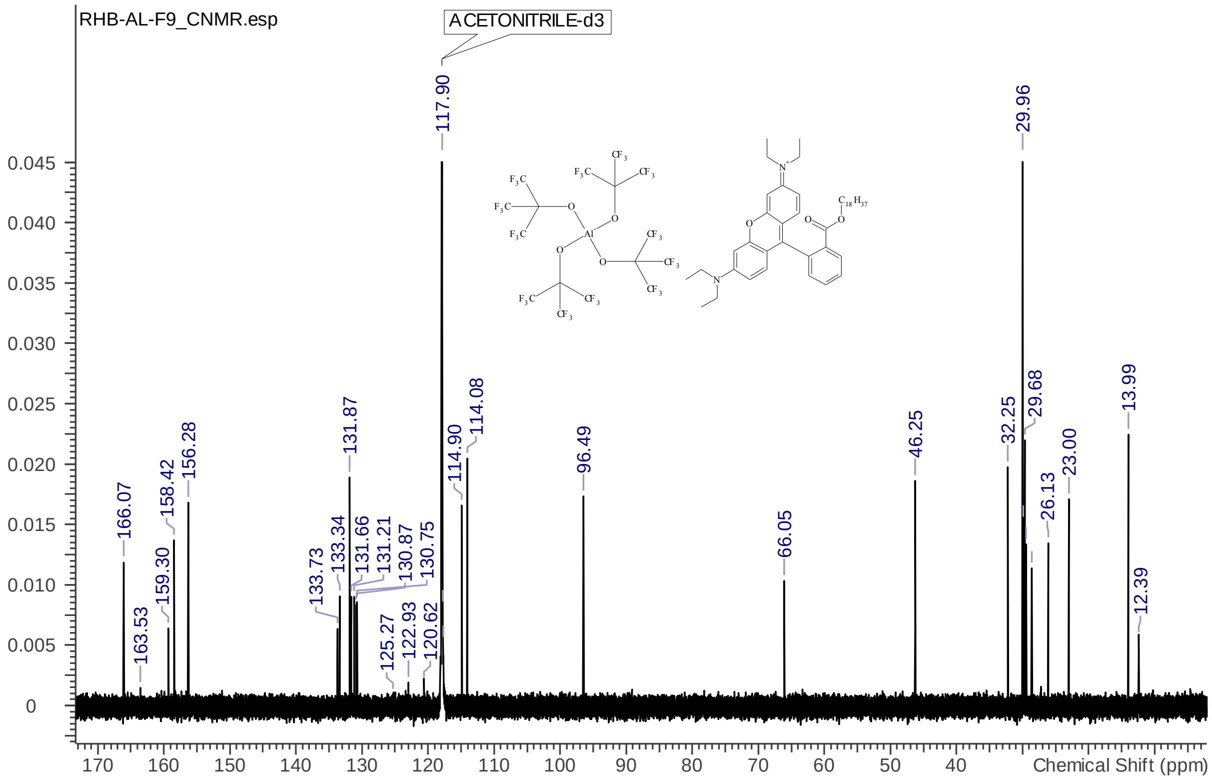


Figure S3. ^{13}C NMR of **R18/F9-Al** in MeCN-d3.

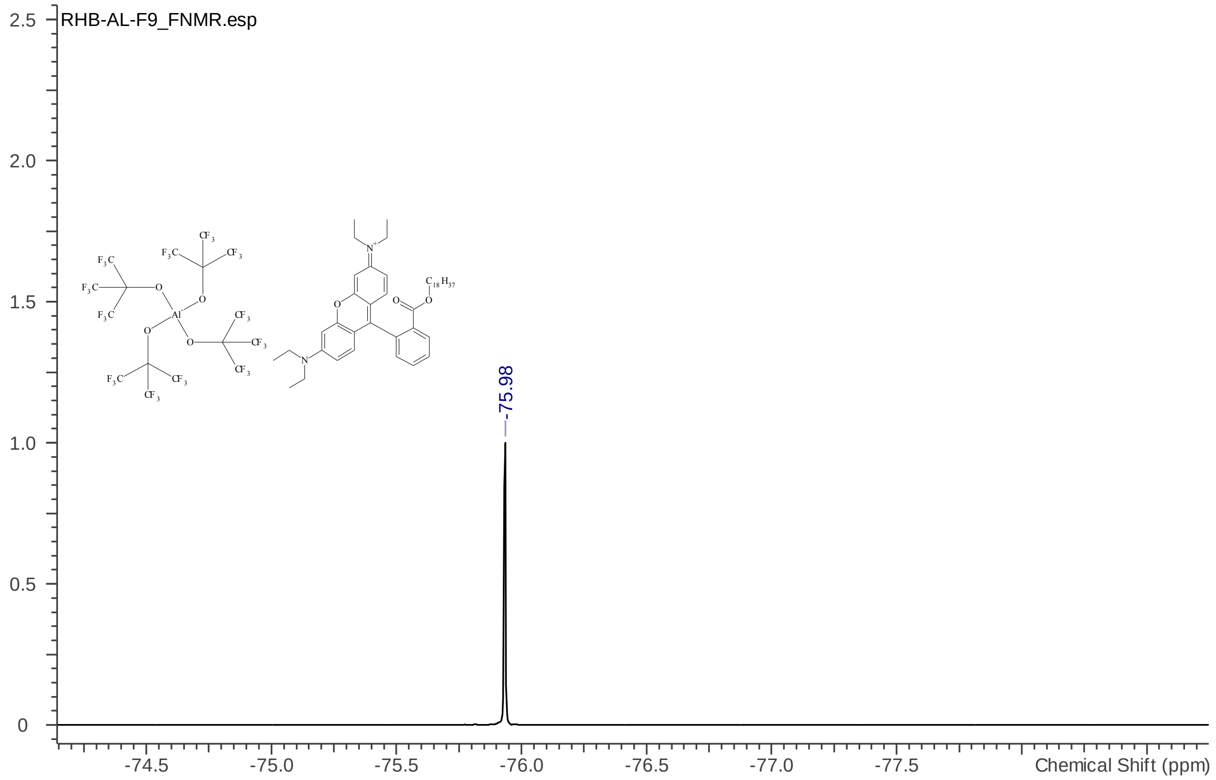


Figure S4. ^{19}F NMR of **R18/F9-Al** in MeCN-d3.

Supplementary figures

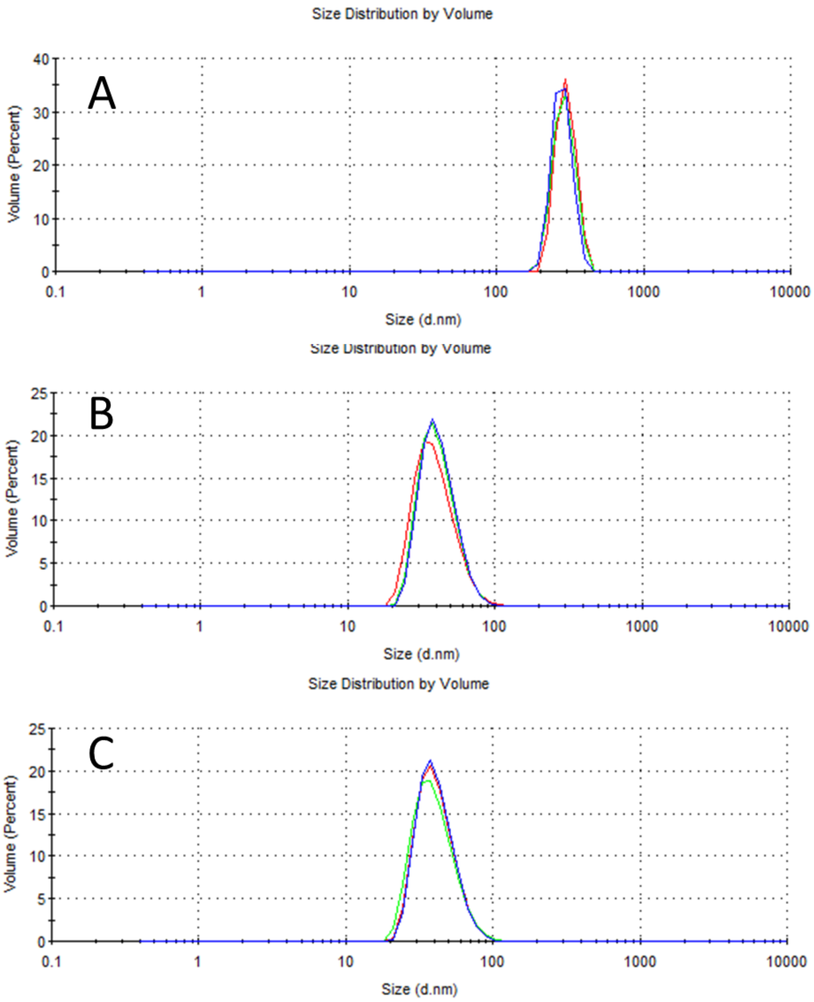


Figure S5. **Size distribution of NPs as measured by DLS.** R18/ClO_4 (A), R18/F5-TPB (B) and R18/F9-Al (C) NPs at 50 mM dye loading. Blue, green and red curves correspond to three consecutive measurements of the same sample.

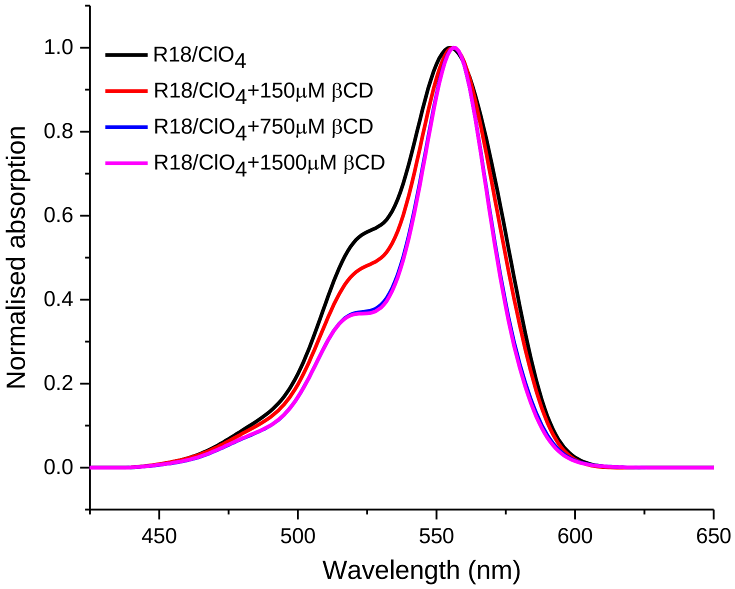


Figure S6. **Effect of β-cyclodextrin** (βCD) **on absorption spectra of R18/ClO$_4$ in water.** Approximately 2 μM solution of R18/ClO$_4$ dye in water was treated with different amounts of β-cyclodextrin. Spectra demonstrate that β-cyclodextrin can help to dissolve aggregates of R18/ClO$_4$ dye in water, and the required concentration is about 1 mM. Dilution due to addition of β-cyclodextrin was less than 10%.

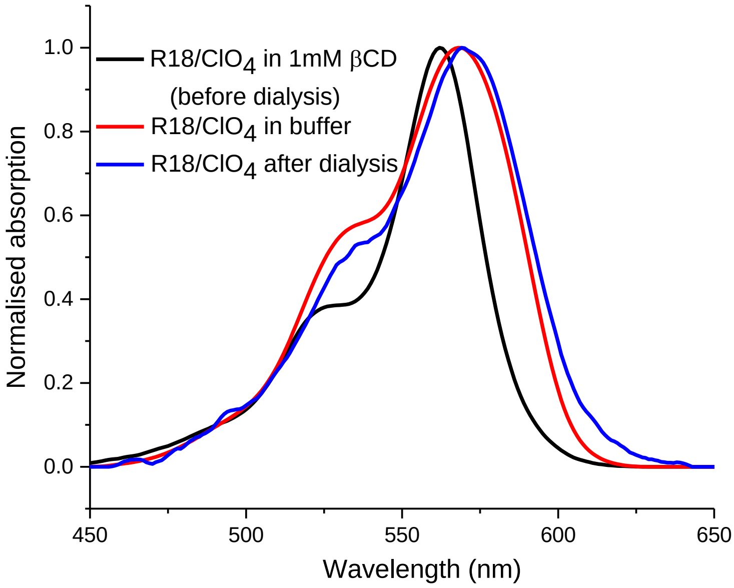


Figure S7. **Normalised absorption spectra of R18/ClO_4 dye in buffer and β-cyclodextrin solution before and after dialysis.** The figure shows, that absorption spectrum of R18/ClO_4 dye after dialysis (blue line) is significantly different from its absorption spectra before dialysis (black line), but very similar to a spectrum of R18/ClO_4 dye in 20 mM phosphate buffer (pH 7.4) (red line). As R18/ClO_4 dye in buffer exists as irregular dye aggregates of different sizes, we can assume that after dialysis of R18/ClO_4 dye solution, traces of remaining dye are in the aggregated state. These aggregates are probably too large to pass through the dialysis membrane.

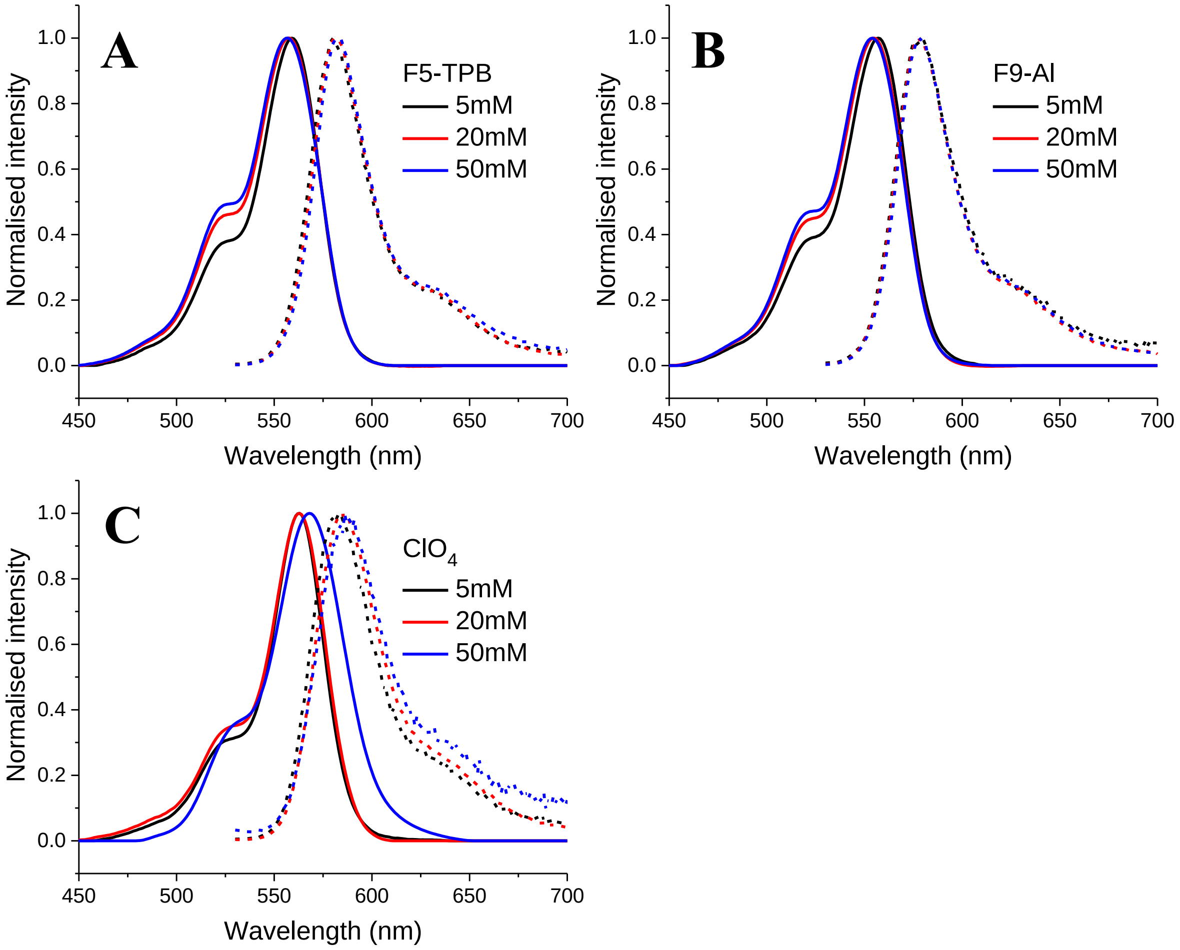


Figure S8. **Effect of dye loading on spectral properties of R18 NPs.** Normalised absorption and fluorescence spectra of R18/F5-TPB (a), R18/F9-Al (b), R18/ClO_4 (c) at different dye loadings.

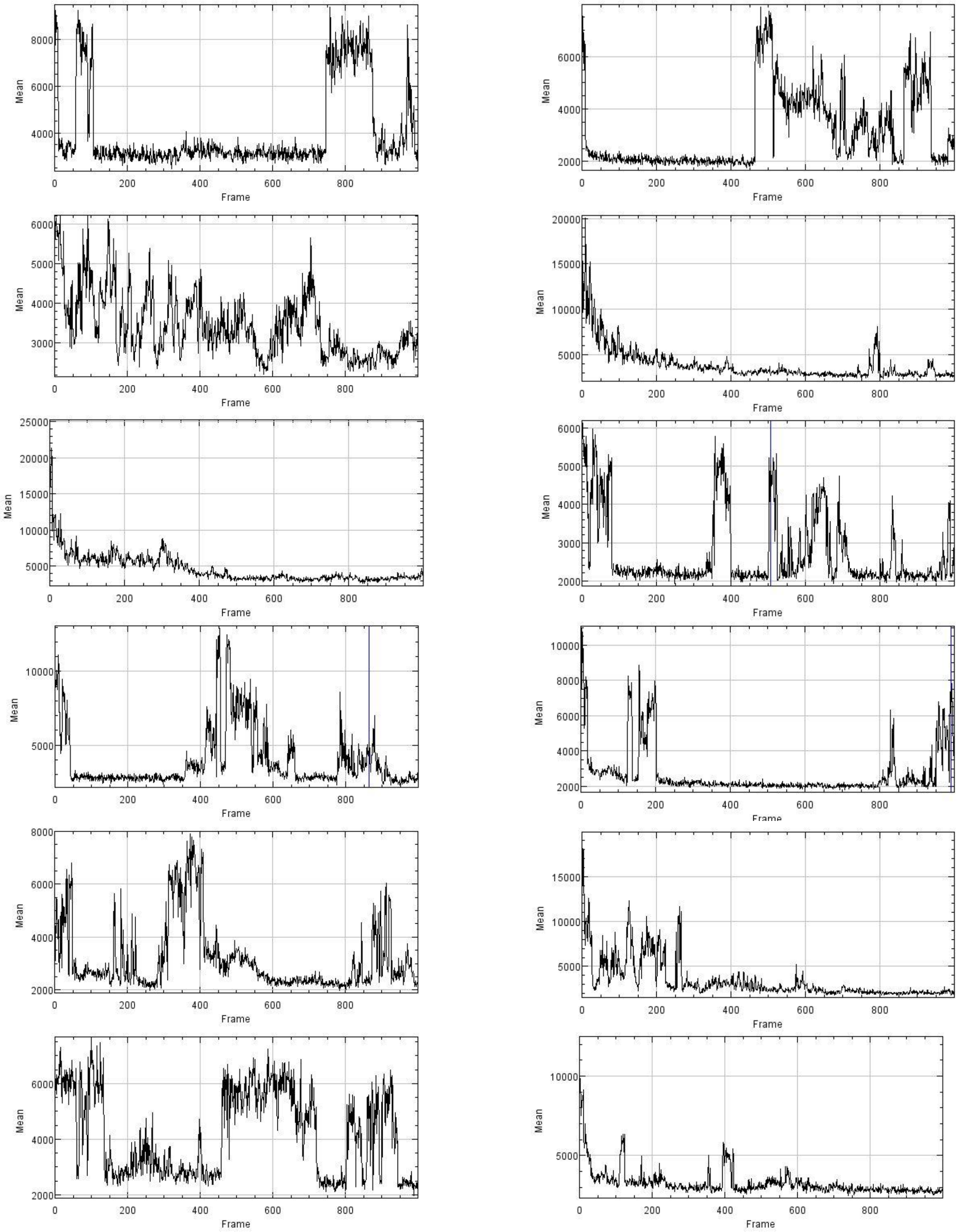


Figure S9. Single-particle blinking. Different emission transients of 50 mM-loaded R18/F5-TPB (right) and R18/F9-Al (left) NPs under 0.6 W/cm^2 532 nm laser illumination. Recording speed was 33 frames per second. No significant difference in blinking behaviour was observed between the two types of NPs.

Supplementary tables

Table S1. Dynamic light scattering data (mean size based on volume statistics, polydispersity, zeta potential) for tested NPs.[a]

NPs	Loading (mM)	Size (nm)	Size SD (nm)	PDI	Zeta potential (mV)	Zeta potential SD (mV)
ClO_4	5	49	1	0.05	-28	1
F5-TPB	5	35	2	0.07	-31	2
F9-Al	5	38	1	0.09	-29	3
ClO_4	20	54	14	0.09	-20	6
F5-TPB	20	41	1	0.12	-31	2
F9-Al	20	37	1	0.07	-31	2
ClO_4	50	276	13	0.12	*	*
F5-TPB	50	41	1	0.08	-33	3
F9-Al	50	41	1	0.08	-34	3
Blank	0	37	1	0.16	-29	3

[a] SD – standard deviation of the mean (n = 3), PDI – polydispersity index.

*Zeta potential for 50 mM R18/ClO_4 NPs could not be measured due to NPs aggregation.

Table S2. Encapsulation efficiency of 50 mM dye-loaded NPs.[a]

NPs	Encapsulation (%)	SD (%)
F9-Al	96.5	2.0
F5-TPB	91.7	4.1
ClO_4	50.6	1.5
ClO_4 no polymer	13.5[b]	1.8

[a] SD – standard deviation of the mean (n = 3).

[b] This value probably corresponds to the amount of large aggregates of pure dye that cannot go through dialysis membrane.

Table S3. Quantum yield data of tested dye-loaded NPs.[a]

NPs	Loading (mM)	Quantum yield	SD
ClO_4	5	0.81	0.01
F5-TPB	5	1.0	0.03
F9-Al	5	0.88	0.01
ClO_4	20	0.21	0.01
F5-TPB	20	0.84	0.01
F9-Al	20	0.71	0.01
ClO_4	50	0.07	0.01
F5-TPB	50	0.55	0.01
F9-Al	50	0.55	0.01

[a] SD – standard deviation of the mean (n = 3).

Table S4. Anisotropy of tested dye-loaded NPs.[a]

NPs	Loading (mM)	Anisotropy	SD
ClO_4	0.5	0.238	0.008
ClO_4	5	0.0892	0.0043
ClO_4	20	0.0351	0.0012
ClO_4	50	0.0250	0.0004
F5-TPB	0.5	0.224	0.003
F5-TPB	5	0.0114	0.0006
F5-TPB	20	0.0008	0.0001
F5-TPB	50	0.0007	0.0001
F9-Al	0.5	0.219	0.009
F9-Al	5	0.0119	0.0030
F9-Al	20	0.0035	0.0001
F9-Al	50	0.0013	0.0002

[a] SD – standard deviation of the mean (n = 2; 10 measurements per sample).

Supplementary references

1. A. Reisch, P. Didier, L. Richert, S. Oncul, Y. Arntz, Y. Mely and A. S. Klymchenko, *Nat. Commun.*, 2014, **5**, 4089.
2. I. Krossing, *Chem. Eur. J.*, 2001, **7**, 490-502.