Fluorinated counterion-enhanced emission of rhodamine aggregates: ultrabright nanoparticles for bioimaging and light-harvesting

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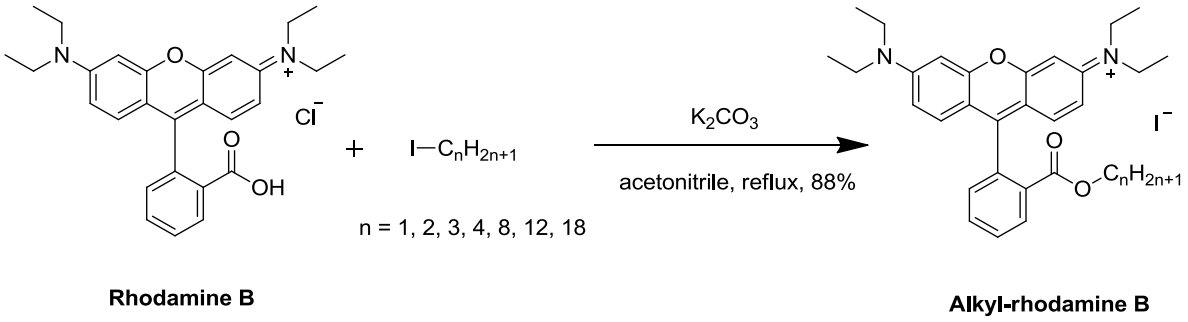
Supporting Information



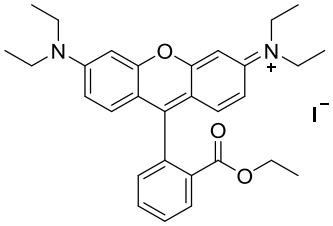
Scheme S1. Synthesis of alkyl-rhodamines B dyes.

General protocol for the synthesis of alkyl-rhodamine B dyes.

Rhodamine B (300 mg, 1 eq.) and potassium carbonate (173 mg, 2 eq.) were placed in a reaction flask, 30 ml of acetonitrile were then added *via* syringe. The vessel was closed with a rubber septum and purged with argon for 5 min. After pre-heating to 60 °C for 30 min the corresponding alkyl iodide (2 eq.) was added dropwise via syringe. The contents of the flask were briefly mixed and stirred under reflux overnight. After cooling to room temperature, 30 mL of acetonitrile were added and the excess of potassium carbonate was filtered off, followed by concentrating the filtrate under vacuum. The crude product was purified by gradient flash column chromatography (SiO_2, DCM/MeOH, from 99:1 to 95:5), which afforded desired product in form of red solid (yield in range of 75-91%) of corresponding Rhodamine B alkyl ester iodide salt**.**

Rhodamine B ethyl ester iodide (R2):

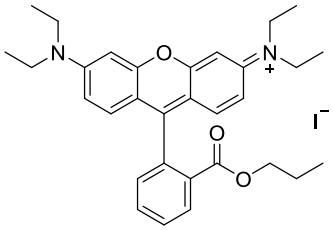
N-(6-(diethylamino)-9-(2-(ethoxycarbonyl)phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide.



Compound was synthesized as described in the general protocol above. Yield 88%. ^{1}H NMR (400 MHz, Chloroform-d, δ): 8.18 (dd, J = 7.9, 1.3 Hz, 1H), 7.72 (td, J = 7.6, 1.4 Hz, 1H), 7.64 (td, J = 7.7, 1.4 Hz, 1H), 7.20 (dd, J = 7.6, 1.2 Hz, 1H), 6.97 (d, J = 9.5 Hz, 2H), 6.81 (dd, J = 9.5, 2.5 Hz, 2H), 6.69 (d, J = 2.4 Hz, 2H), 3.96 (q, J = 7.1 Hz, 2H), 3.55 (q, J = 7.2 Hz, 8H), 1.22 (t, J = 7.1 Hz, 12H), 0.96 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 164.78, 158.66, 157.45, 155.26, 133.14, 132.80, 131.04, 130.16, 129.88, 114.10, 113.26, 96.01, 61.26, 46.03, 13.59, 12.51; UV–vis (*methanol*): λ_{max} = 560 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for $C_{30}H_{35}N_2O_3$, 471.26422; found , 471.26426.

Rhodamine B n-propyl ester iodide (R3):

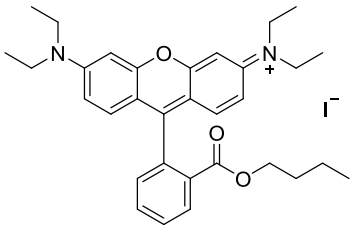
N-(6-(diethylamino)-9-(2-(propoxycarbonyl)phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide



Compound was synthesized as described in the general protocol above. Yield 89%. ^{1}H NMR (400 MHz, Chloroform-*d*, δ): 8.26 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.79 (td, *J* = 7.6, 1.4 Hz, 1H), 7.71 (td, *J* = 7.7, 1.4 Hz, 1H), 7.28 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.05 (d, *J* = 9.4 Hz, 2H), 6.87 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.77 (d, *J* = 2.5 Hz, 2H), 3.95 (t, *J* = 6.7 Hz, 2H), 3.62 (q, *J* = 7.1 Hz, 8H), 1.44 (h, *J* = 7.1 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 12H), 0.75 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.18, 158.99, 157.77, 155.55, 133.46, 133.09, 131.35, 131.33, 130.43, 130.24, 130.21, 114.34, 113.58, 96.36, 67.26, 46.31, 21.73, 12.77, 10.32; UV–vis (*methanol*): λ_{max} = 560 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for C31H37N2O3, 485.27987; found, 485.27953.

Rhodamine B n-butyl ester iodide (R4):

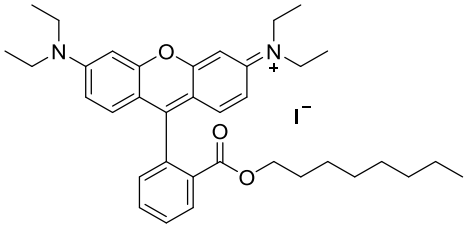
N-(9-(2-(butoxycarbonyl)phenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide



Compound was synthesized as described in the general protocol above. Yield 77%. ^{1}H NMR (400 MHz, Chloroform-*d*, δ): 8.23 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.77 (td, *J* = 7.5, 1.4 Hz, 1H), 7.69 (td, *J* = 7.7, 1.4 Hz, 1H), 7.25 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.02 (d, *J* = 9.5 Hz, 2H), 6.86 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.74 (d, *J* = 2.4 Hz, 2H), 3.95 (t, *J* = 6.5 Hz, 2H), 3.60 (q, *J* = 7.2 Hz, 8H), 1.34 (p, *J* = 14.7, 6.8 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 12H), 1.09 (h, *J* = 7.4 Hz, 2H), 0.74 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.16, 158.84, 157.69, 155.49, 133.26, 132.99, 131.28, 130.36, 130.19, 130.13, 114.29, 113.49, 96.25, 65.48, 46.24, 30.30, 19.00, 13.61, 12.70; UV–vis (*methanol*): λ_{max} = 560 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for C32H39N2O3, 499.29552; found, 499.29529.

Rhodamine B n-octyl ester iodide (R8):

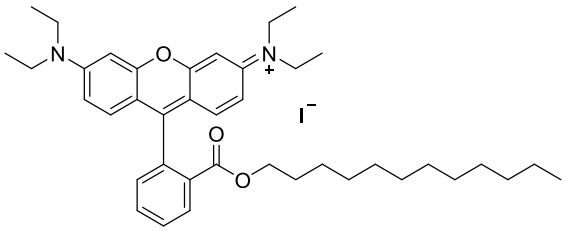
N-(6-(diethylamino)-9-(2-((octyloxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide



Compound was synthesized as described in the general protocol above. Yield 79%. ^{1}H NMR (400 MHz, Chloroform-*d*, δ): 8.20 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.74 (td, *J* = 7.5, 1.4 Hz, 1H), 7.66 (td, *J* = 7.7, 1.3 Hz, 1H), 7.22 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.99 (d, *J* = 9.5 Hz, 2H), 6.83 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.71 (d, *J* = 2.4 Hz, 2H), 3.91 (t, *J* = 6.6 Hz, 2H), 3.58 (q, *J* = 7.2 Hz, 8H), 1.32 (p, *J* = 6.8 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 12H), 1.20 – 0.97 (m, 10H), 0.76 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.03, 158.76, 157.56, 155.36, 133.17, 132.92, 131.17, 131.16, 130.26, 130.04, 130.02, 114.18, 113.36, 96.11, 65.61, 46.12, 31.57, 28.94, 28.89, 28.13, 25.61, 22.42, 13.93, 12.60; UV–vis (*methanol*): λ_{max} = 560 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for C36H47N2O3, 555.35812; found, 555.35832.

Rhodamine B dodecyl ester iodide (R12):

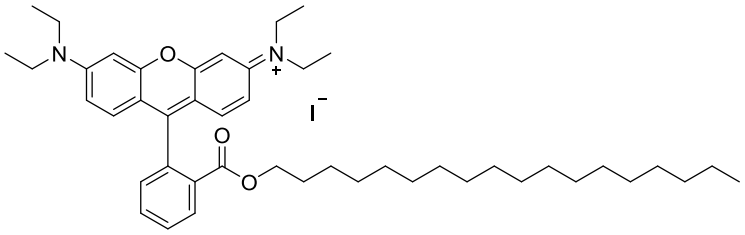
N-(6-(diethylamino)-9-(2-((dodecyloxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide



Compound was synthesized as described in the general protocol above. Yield 82%. ^{1}H NMR (400 MHz, Chloroform-*d*, δ): 8.20 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.75 (td, *J* = 7.6, 1.4 Hz, 1H), 7.67 (td, *J* = 7.7, 1.3 Hz, 1H), 7.23 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.00 (d, *J* = 9.5 Hz, 2H), 6.84 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.72 (d, *J* = 2.4 Hz, 2H), 3.92 (t, *J* = 6.6 Hz, 2H), 3.58 (q, *J* = 7.2 Hz, 8H), 1.33 (p, *J* = 6.7 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 12H), 1.21 – 0.99 (m, 18H), 0.78 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.04, 158.80, 157.59, 155.39, 133.21, 132.94, 131.20, 131.18, 130.28, 130.07, 130.04, 114.20, 113.40, 96.14, 65.65, 46.14, 31.74, 29.47, 29.41, 29.28, 29.17, 29.03, 28.17, 25.66, 22.52, 13.98, 12.62; UV–vis (*methanol*): λ_{max} = 561 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for C40H55N2O3, 611.42072; found, 611.42082.

Octadecyl Rhodamine B iodide (R18):

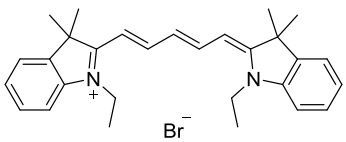
N-(6-(diethylamino)-9-(2-((octadecyloxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium iodide



The general protocol above was modified by using DMF as a solvent. Then, after removal the excess of potassium carbonate by filtration, mature solution was concentrated under vacuum, redissolved in 50mL of DCM and washed three times with brine, then organic layer was separated, dried over sodium sulphate and evaporated. Purification was done by flash column chromatography as described above. Yield 80%. ^{1}H NMR (400 MHz, Chloroform-*d*, δ): 8.24 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.79 (td, *J* = 7.5, 1.4 Hz, 1H), 7.70 (td, *J* = 7.7, 1.4 Hz, 1H), 7.28 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.04 (d, *J* = 9.5 Hz, 2H), 6.87 (dd, *J* = 9.5, 2.5 Hz, 2H), 6.76 (d, *J* = 2.4 Hz, 2H), 3.96 (t, *J* = 6.6 Hz, 2H), 3.62 (q, *J* = 7.2 Hz, 8H), 1.37 (p, *J* = 6.8 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 12H), 1.25 – 0.96 (m, 30H), 0.83 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.16, 158.96, 157.74, 155.54, 133.36, 133.05, 131.33, 131.29, 130.39, 130.22, 114.34, 113.57, 96.32, 65.79, 46.27, 31.90, 29.67, 29.62, 29.57, 29.43, 29.33, 29.18, 28.32, 25.80, 22.66, 14.09, 12.74; UV–vis (*methanol*): λ_{max} = 564 nm; HRMS (ESI) *m*/*z*: $[M]^+$ calcd for C46H67N2O3, 695.51462; found, 695.51569.

1,1'-diethyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate (C2-Cy5):

1-ethyl-2-((1E,3E)-5-((Z)-1-ethyl-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium bromide



Synthesis of C2-Cy5 was done as described elsewhere.[1]

Characterization of ion-associated NPs

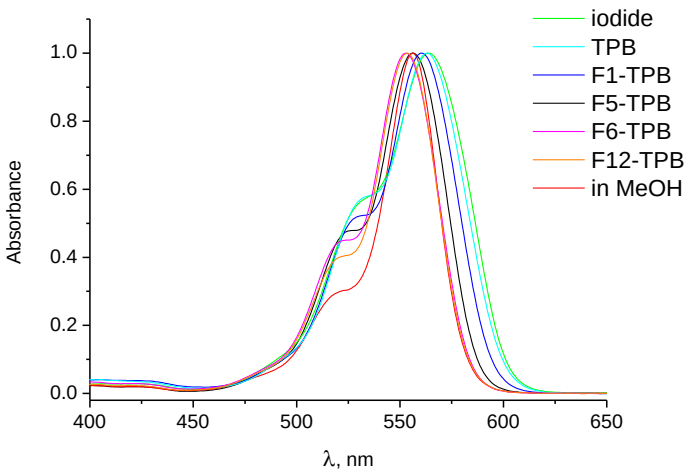


Figure S1. Normalized absorption spectra of R18 dye with different TPB counterions and comparison to R18 iodide in water and methanol.

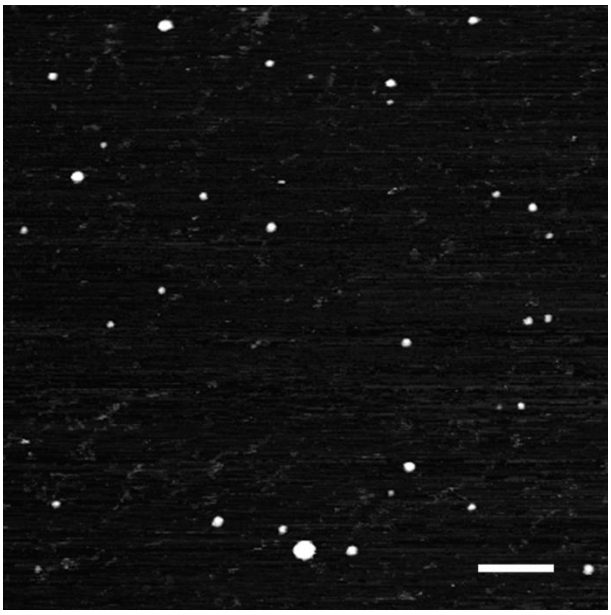


Figure S2. Atomic force microscopy of nanoparticles formed by R12 with F6-TPB counterion. The imaging was done liquid with 10-100 mM $CaCl_2$ (B). Scale bar is 500 nm. Traces in this image were observed because some particles were not sufficiently well immobilized and moved during scan.

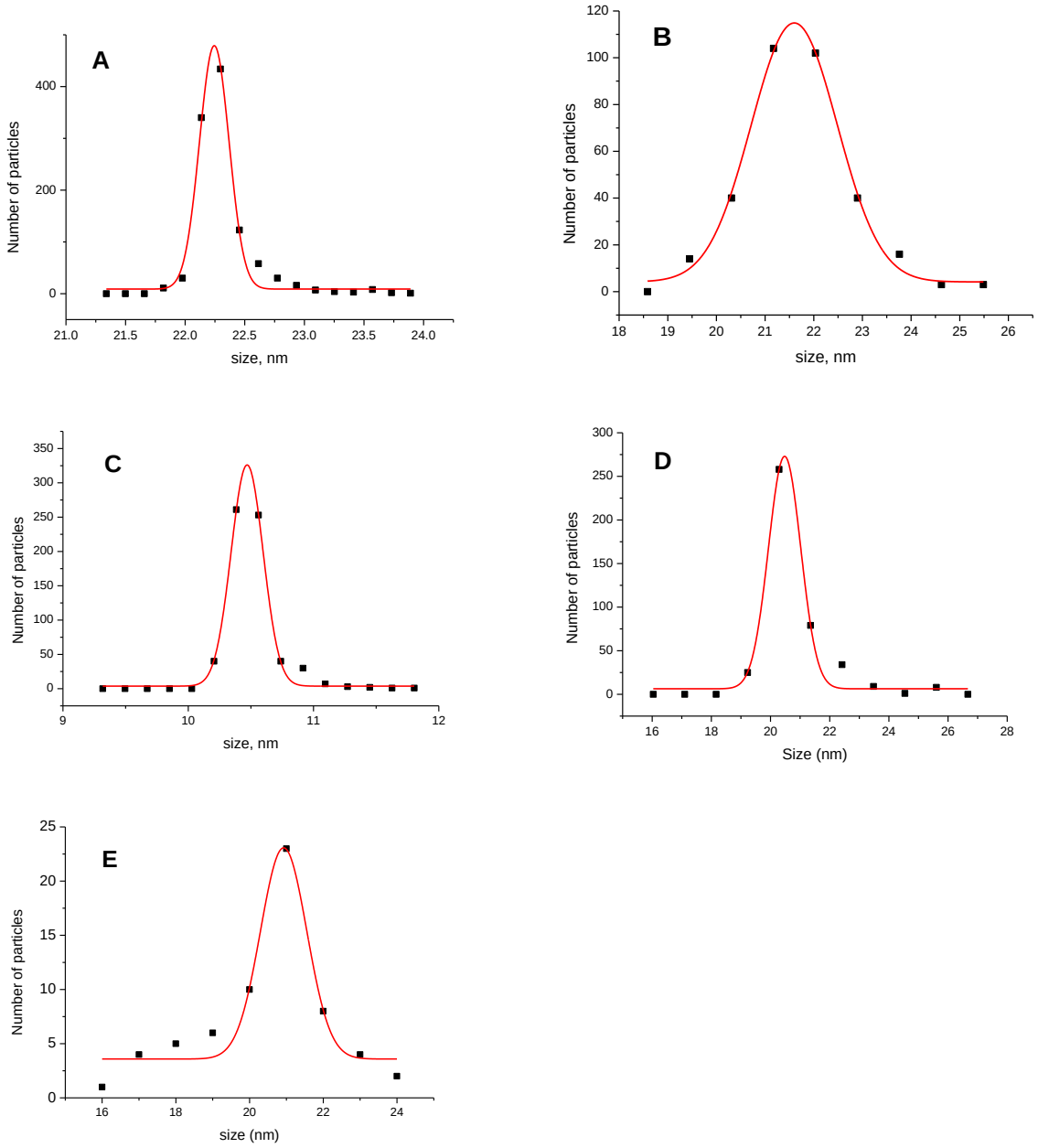


Figure S3. AFM size statistics of NPs prepared from R12 with different counterions: (A) TPB, average size 22 nm; (B) F1-TPB, average size 22 nm; (C) F5-TPB, average size 11 nm; (D) F6-TPB, average size 21 nm; (E) F12-TPB, average size 21 nm. Particle diameter was estimated from the height measurements.

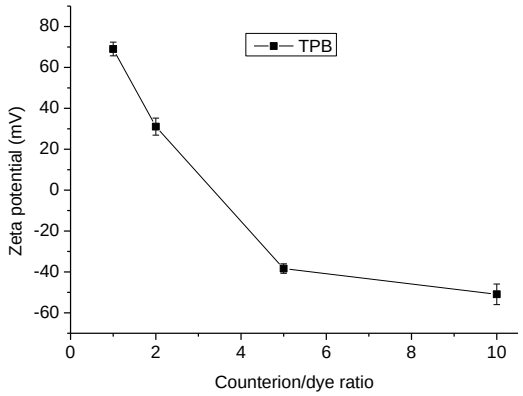


Figure S4. Zeta potential of nanoparticles formed by R12 with non-fluorinated counterion TPB. Dye concentration was 20 μM.

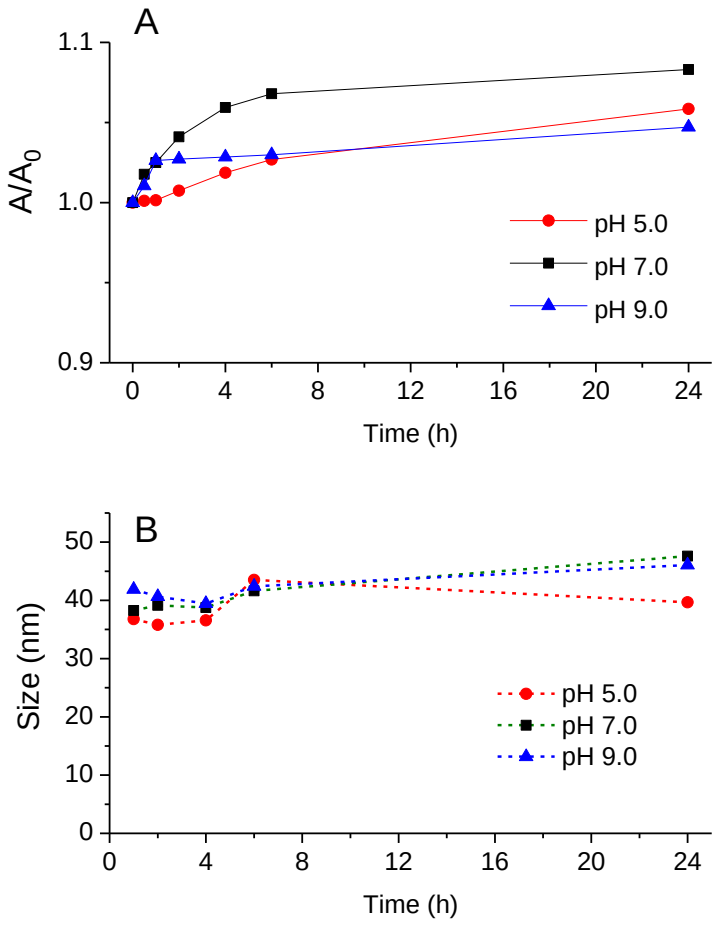


Figure S5. Stability of R12/F12-TPB NPs at different pH, measured by changes by absorption spectroscopy and DLS. A/A_0 is absorbance at the maximum for a sample at a given time divided by that at time zero. Statistics by volume was used in DLS measurements. Measurements were done in plastic cuvettes; concentrations of R12 and F12-TPB were 1 µM and 10 µM, respectively. To control pH, the following buffers were used: 5 mM MES buffer (pH 5) and 5 mM TRIS buffer (pH 7 or 9).

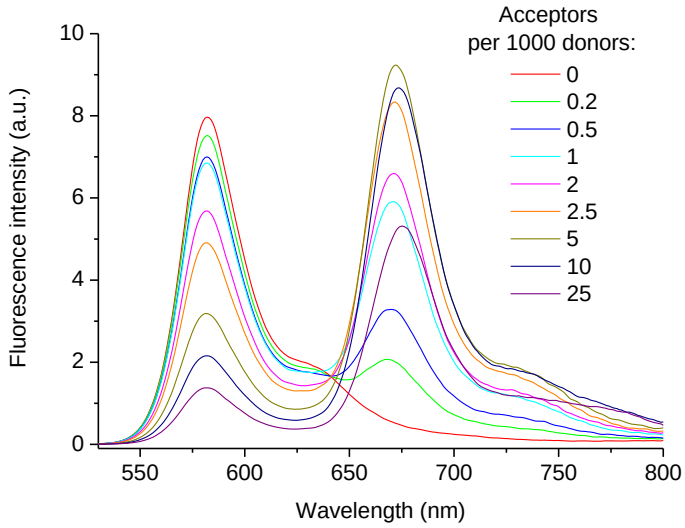


Figure S6. FRET inside R12/F5-TPB NPs. Fluorescence spectra of R12 NPs with F5-TPB counterions containing different amounts of C2-Cy5 acceptor dye. Excitation wavelength was 520 nm.

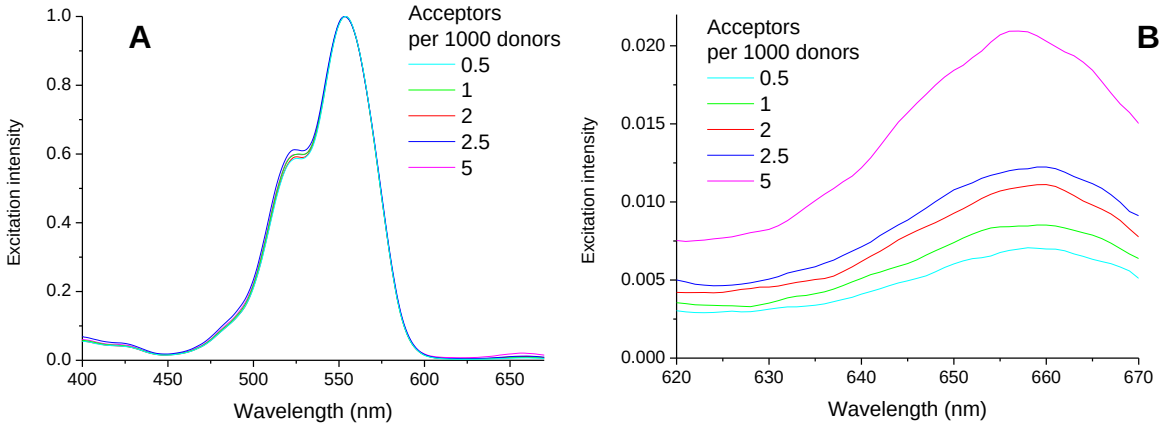


Figure S7. Excitation spectra describing the antenna effect. (A) Normalized excitation spectra of R12 NPs with F5-TPB counterion containing different amounts of C2-Cy5 acceptor dye. (B) Zoomed region of the excitation spectra focused on the excitation of the acceptor. Emission was detected at 680 nm.

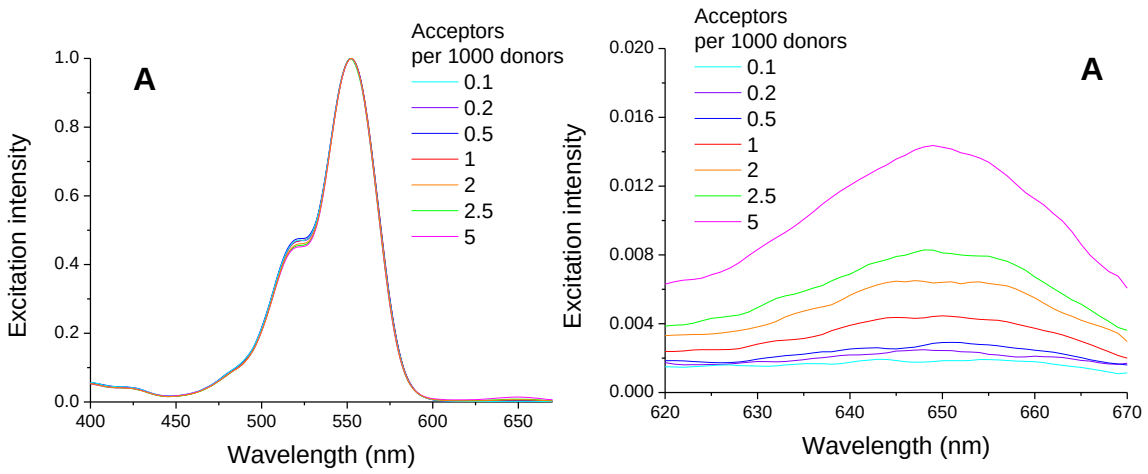


Figure S8. Excitation spectra describing the antenna effect. (A) Normalized excitation spectra of R12 NPs with F12-TPB counterion containing different amounts of C2-Cy5 acceptor dye. (B) Zoomed region of the excitation spectra focused on the excitation of the acceptor. Emission was detected at 680 nm.

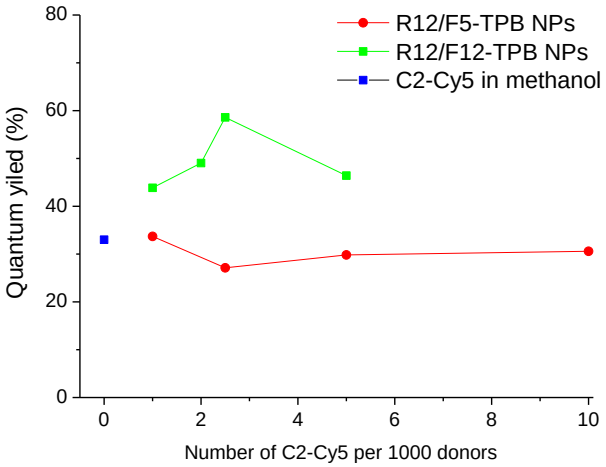


Figure S9. Fluorescence quantum yield of C2-Cy5 dye inside R12 NPs with F5-TPB and F12-TPB counterions in comparison to that in methanol.

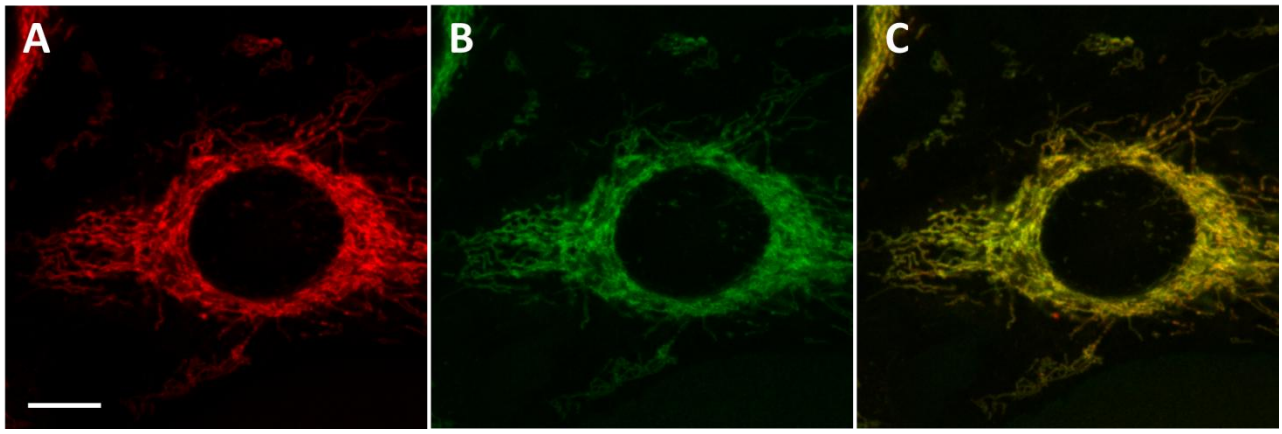


Figure S10. Fluorescence imaging of a HeLa cell in the presence of R12 dye after 1h of incubation at 37 °C (A). (B) The same cell is stained with Mito-Tracker® green. (C) Merged image of green and red channels showing perfect co-localization of the two dyes. Fluorescence images were taken on a Leica TSC SPE confocal microscope. The microscope settings were: 561 nm laser source with 567-700 nm detection range for ion-associated NPs and 488 nm excitation with 503-550 nm emission range for Mito-Tracker® green. Scale bar is 10 µM.

Literature:

[1] D. S. Pisoni, L. Todeschini, A. C. A. Borges, C. L. Petzhold, F. S. Rodembusch, L. F. Campo, *J. Org. Chem.* **2014**, *79*, 5511.