Supporting Information for

Control of the fluorescence lifetime in dye based nanoparticles

Stine G. Stenspil,[a] Junsheng Chen,[a] Mikkel B. Liisberg,[a] Amar H. Flood,*[b] Bo W. Laursen*[a]

[a] Nano-Science Center & Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 København Ø, Denmark

[b] Department of Chemistry, Indiana University, 800 East Kirkwood Avenue, Bloomington, Indiana 47405, United States

* Corresponding authors e-mail: bwl@chem.ku.dk, aflood@indiana.edu

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Materials

3,3'-Dihexyloxacarbocyanine iodide (DiOC6) was purchased from Alfa Aesar. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly (ethylene glycol-2000) (DSPE-PEG) was purchased from Biochempeg. Milli-Q water (18.2MΩ) was obtained using a Millipore purification system. Cyanostar and dioctyl-DAOTA BF_4^- was synthesized based on previously reported procedures.[1-2] R12 was obtained by modifying rhodamine B with a lipophilic chain with a method reported by Klymchenko and co-workers.[3-4]

DiOC6 was received as an iodide salt, therefore, a counterion exchange to hexafluorophosphate was needed. It was done by adding an acetonitrile solution of DiOC6 iodide to an aqueous solution of potassium hexafluorophosphate 0.2M under vigorous stirring. The resulting precipitate was collected by filtration. This procedure was repeated twice in order to ensure complete ion exchange.

SMILES NPs preparation

The SMILES NPs were formed using a previously reported nanoprecipitation technique.[4] A THF precursor solution was prepared with the cationic fluorophores (DiOC6, R12 and/or DAOTA), 2.5 mol eq. CS, and 56 wt% amphiphilic surface capping agent 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethyleneglycol-2000 (DSPE-PEG). The precursor solution (0.4 mL) was then injected into excess MilliQ water (10 mL) while under sonication to rapidly disperse the solution and form NPs. The sonication was kept on for 5 min to ensure complete dispersion. SMILES NPs for lifetime imaging were prepared using the same procedure but without DSPE-PEG.

Spectroscopic characterization

Absorption spectra were measured on an Agilent Cary 300 spectrometer.

Steady-state fluorescence spectra and fluorescence decays were measured on a FluoTime 300 fitted with a cw-xenon lamp from Picoquant.

Pulsed diode laser heads LDH-P-C-470 and LDH-D-TA-530B from Picoquant were used to record fluorescence decays. The decay histograms were analyzed using FluoFit software by applying the exponential reconvolution fitting method.

Fluorescence quantum yields were recorded relative to fluorescein in 0.1 M NaOH (QY=0.9).[5]

Dynamic light scattering (DLS)

DLS was carried out on a Malvern Zetasizer with a HeNe 633 nm laser. Before DLS measurements, particle solutions were filtered twice through a hydrophilic PTFE filter (0.22 µm) in order to reduce scattering from dust. Each sample was measured with backscattering

at 179˚ and repeated 3-5 times, the average, standard deviation of the mean and polydispersity index of these measurements are stated in the tables below.

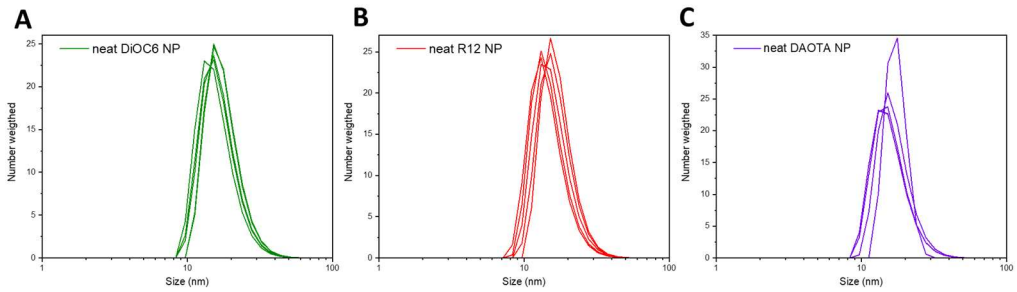

Figure S1 Number weighted size distributions of neat NPs. A) DiOC6 NPs, B) R12 NPs, and C) DAOTA NPs. (5 repeat measurements)

Table S1 Average of the mean number weighted size of neat NPs with standard deviation (n=5) and average polydispersity index.

NP	**Number weighted Average size (nm)**	**PDI**
DiOC6	16±1	0.20
R12	17±1	0.17
DAOTA	17±1	0.18

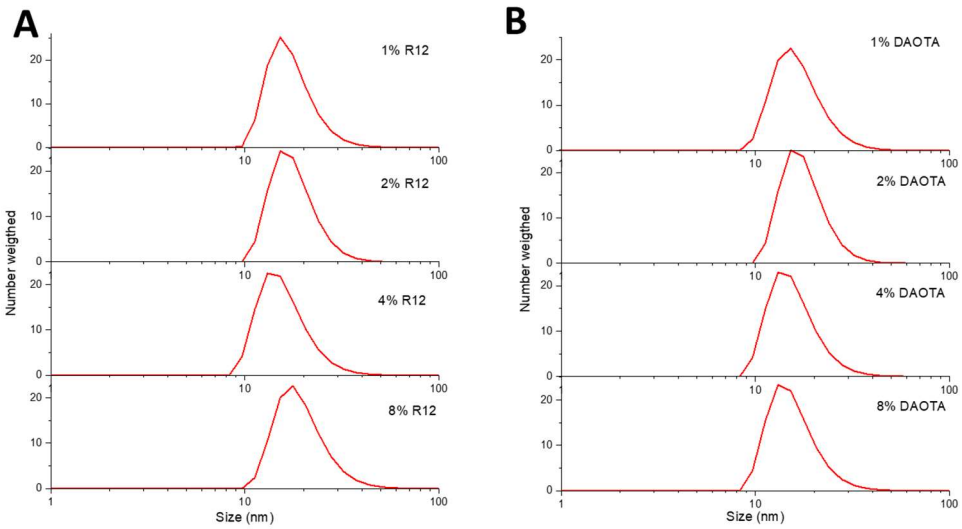

Figure S2 Number weighted size distribution of NPs doped with either A) R12 or B) DAOTA in the range of 1-8 mol%.

Table S2 Average of the mean number weighted size with standard deviation and polydispersity of doped NPs (n=3).

mol%	DiOC6:R12, Size (nm)	PDI	DiOC6:DAOTA, Size (nm)	PDI
1	17±1.9	0.25	17±0.2	0.32
2	18±2.4	0.20	17± 2	0.26
4	17±1.1	0.25	17±2.1	0.28
8	21±1.3	0.25	17±1.3	0.26

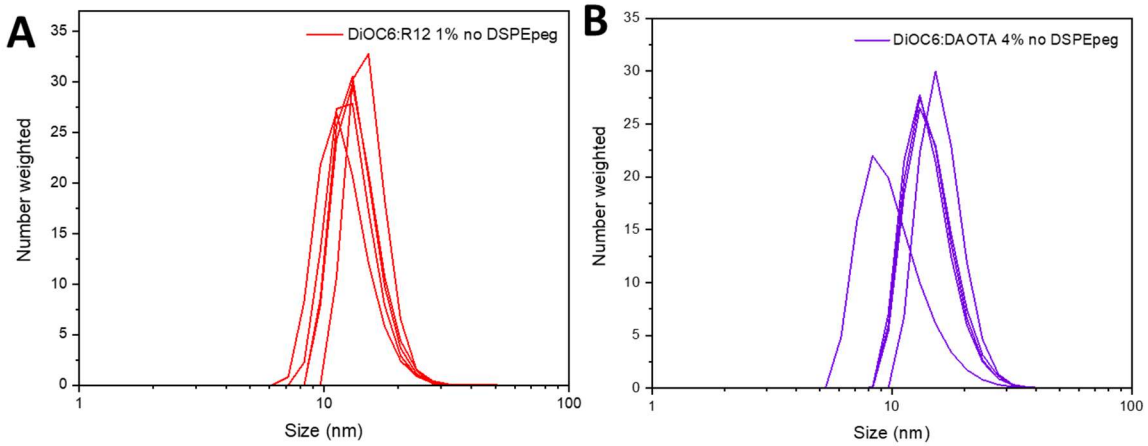

Figure S3 Number weighted DLS of doped NPs without DSPE-PEG added .A) DiOC6 1 %mol R12 and B) DiOC6 4 %mol DAOTA. (5 repeat measurements)

Table S3 Average of the mean number weighted size with standard deviation and polydispersity of non-coated NPs used for lifetime imaging, (n=5).

	Size (nm)	PDI
DiOC6 4% DAOTA	14±2	0.34
DiOC6 1% R12	12±3	0.37

Properties of neat SMILES NPs

Table S4 Photophysical properties of fluorophores in DCM compared to SMILES NPs.

	$\phi_{fl.}$ **in DCM**[a]	τ_{fl} **in DCM (ns)**	$\phi_{fl.}$ **NPs**[b]	τ **NPs (ns)**	**Decay measured at: (nm)**
DiOC6	0.06	0.26 (54%) 0.08 (46%) **0.18**	0.13	0.94 (44%) 0.21 (16%) 2.38 (41%) **1.41**	550
R12	0.73	3.77	0.06	0.31 (31%) 1.21 (43%) 3.04 (27%) **1.42**	700
DAOTA	0.80	21.3	0.34	26.3 (90%) 12.2 (10%) **24.8**	700

[a] QY in DCM from following references Kacenauskaite et al.[6] and Bogh et al.[7] [b]QY of NPs relative to the corresponding fluorophores in DCM.

Optical properties fluorophores

Table S5 Optical properties of dyes in DCM unless otherwise stated.

	$\lambda_{max,abs}$ **(nm)**	$\lambda_{max,fl}$ **(nm)**	ε **at** λ_{max}[b] **($M^{-1}cm^{-1}$)**	ϕ_{fl}[c] **in DCM**	τ_{fl} **(ns)**
$[CS_2PF_6]^-$	323[a]	405[a]	~200000	n.a.	n.a.
DiOC6	489	503	165000	0.06	0.18*
R12	557	575	120000 (THF) 97000 (in NP)	0.73	3.77
DAOTA	560	576	14800	0.80	21.3

[a]Absorption and emission maxima from Chen et al.[4] [b]Absorption coefficients from following references Chen et al.[4, 8] and Laursen et al.[2] [c]QY in DCM from following references Kacenauskaite et al.[6] and Bogh et al.[7] *Intensity weighted average.

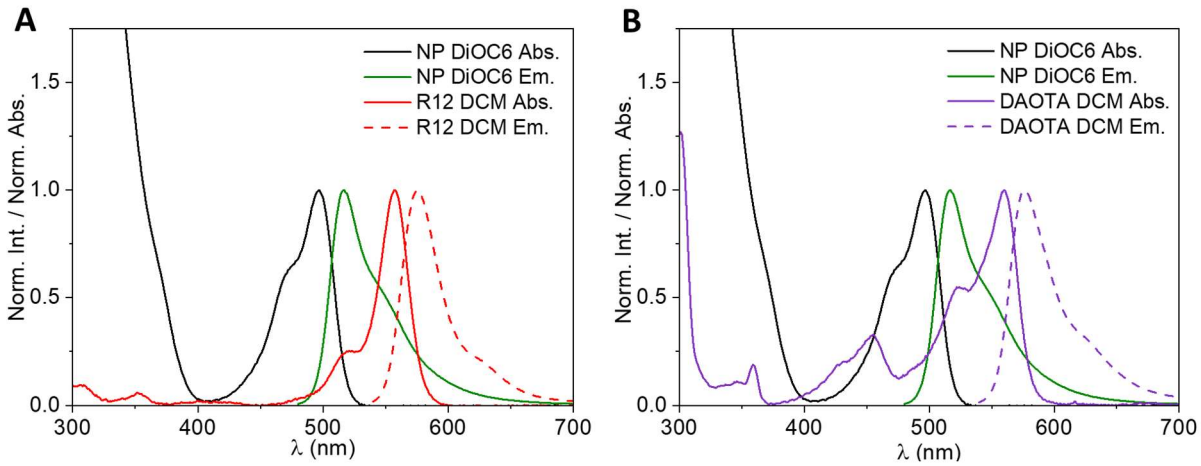

Figure S4 Normalized absorption and emission spectra of NP DiOC6 and A) R12 or B) DAOTA in DCM.

Doped SMILES NPs

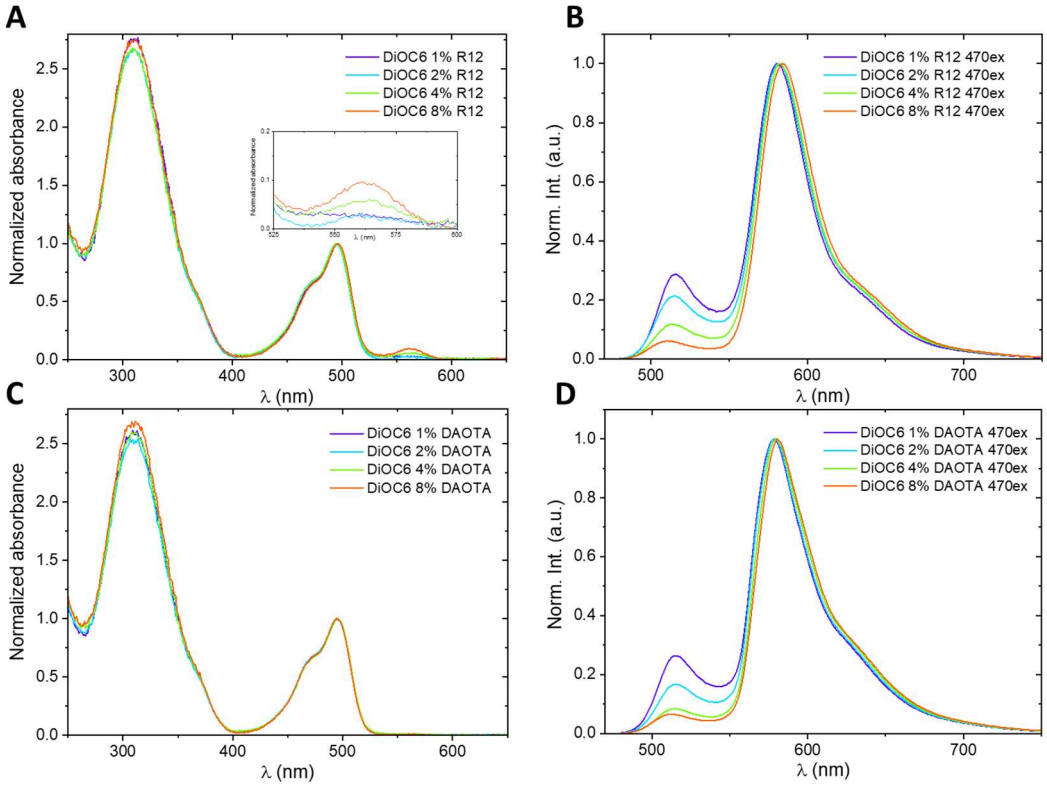

Figure S5 A) Normalized absorption with inset of R12 absorption and B) emission spectra of DiOC6 doped with increasing %mol R12. C) Normalized absorption and D) emission spectra of DiOC6 doped with increasing %mol DAOTA.

Fluorescence decays doped SMILES NPs

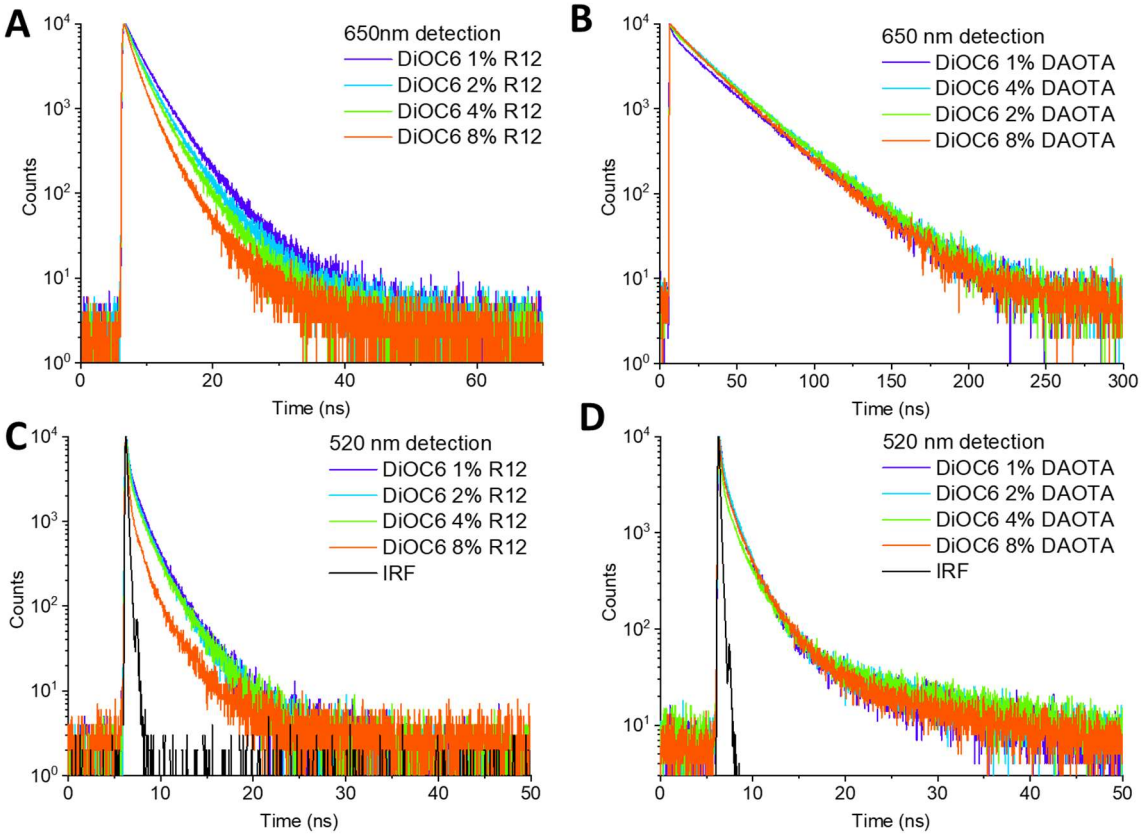

Figure S6 Fluorescence decays for doped DiOC6 NPs with A) R12 and B) DAOTA $\lambda_{em\ det}$= 650 nm and C) R12 and D) DAOTA $\lambda_{em\ det}$= 520 nm all with λ_{ex}=470 nm.

The fluorescence decays measured in the donor band at 520 nm show little variation upon addition of acceptor dyes. This suggest that the residual emission observed from the DiOC6 may originate from domains or particle with low or now acceptor dyes present. In regions containing acceptor dyes, the energy transfer process is so efficient that it is faster than the time resolution on the instrument used.

In all cases, decays were fitted with 2-3 components. However, considering the inhomogeneity of the sample (discussed in the manuscript) interpretation of the individual components and amplitudes would not be reliable. We therefore base our discussion only on the average intensity weighted lifetimes.

Table S6 Intensity weighted fluorescence lifetimes of 1-8% doped NPs detected in the antenna emission band at 520nm and at the dopant emission band at 650nm. The lifetime in bold is the intensity weighted average.

DiOC6:R12	$\tau_{int,520\ nm}$ (ns)	$\tau_{int,650\ nm}$ (ns)	DiOC6: octDAOTA	$\tau_{int,520\ nm}$ (ns)	$\tau_{int,650\ nm}$ (ns)
99:1	0.19 (22%) 2.88 (21%) 1.05 (57%) **1.25**	4.43 (48 %) 2.03 (52%) **3.19**	99:1	1.65 (57%) 0.48 (33%) 12.6 (10%) **2.30**	28.66(81%) 13.2 (17%) 1.15 (2%) **25.55**
98:2	1.23 (58%) 0.35 (18%) 3.14 (24%) **1.53**	2.85 (71%) 1.07 (17%) 7.95 (13 %) **3.19**	98:2	8.49 (25%) 1.21(75%) **2.31**	30.0 (74%) 16.4 (25%) 1.55(1.45%) **26.23**
96:4	2.51 (44%) 0.84 (56%) **1.58**	2.46 (68%) 1.02 (19%) 5.66 (13%) **2.59**	96:4	9.99 (18%) 1.31 (82%) **2.92**	30.0 (68%) 16.65 (21%) 1.92 (1%) **25.65**
92:8	2.64 (34%) 0.78 (66%) **1.41**	1.42 (62%) 0.45 (10%) 3.39 (28%) **1.89**	92:8	4.11 (49%) 1.09 (51%) **2.03**	26.68 (77%) 11.76 (23%) **23.25**

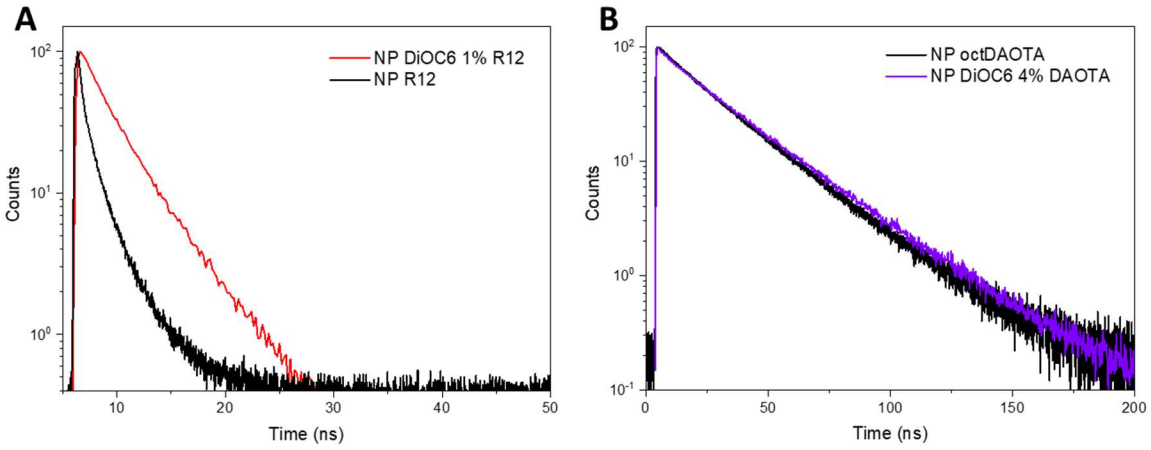

Figure S7 Decays of doped NPs compared to neat NPs. A) R12 and B) DAOTA. λ_{ex}=530 nm, $\lambda_{em,det}$= 650 nm.

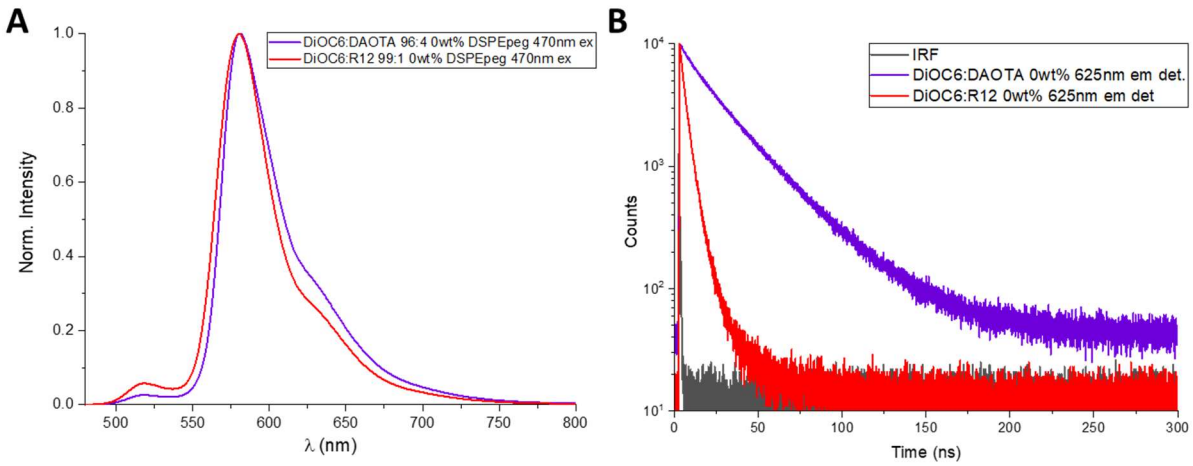

Figure S8 A) Normalized emission and B) fluorescence decays of DiOC6 doped NPs with 1 mol% R12 ($\langle\tau\rangle_{int} = 4.0\ ns$) or 4 mol% DAOTA ($\langle\tau\rangle_{int} = 26.3\ ns$) without DSPE-PEG added.

Quantum yield determination doped SMILES NPs

Fluorescence quantum yields were determined by measuring absorption and emission spectra at a minimum of 6 different concentrations where the absorption is kept under 0.1 for the long wavelength absorption band. Fluorescein was used as the reference dye. The quantum yields were calculated using following equation:

$$\Phi_x = \frac{F_x \cdot f_{ref}}{F_{ref} \cdot f_x} \cdot \frac{n_x^2}{n_{ref}^2} \cdot \Phi_{ref}$$

Where $f = 1 - 10^{-A}$ (using the absorption at the excitation wavelength 470nm), F is the integrated emission and n the refractive index of the solvent.

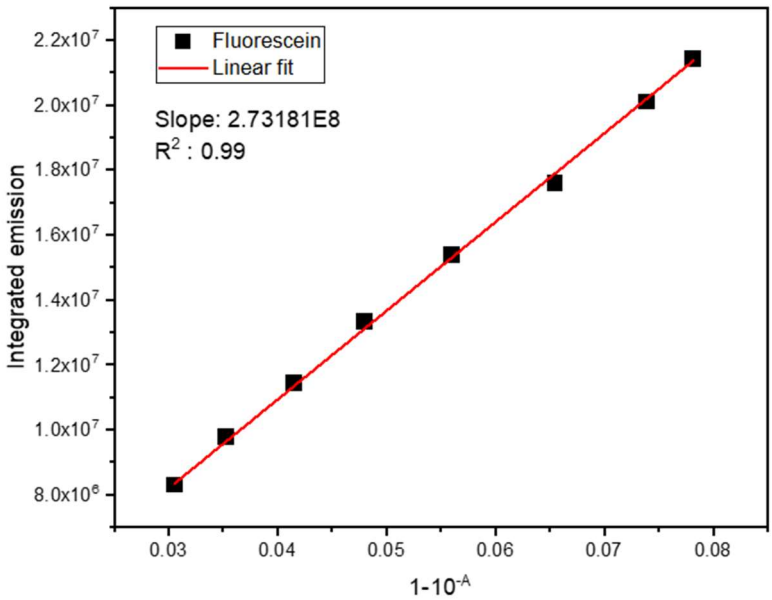

Figure S9 Linear fit of integrated emission as function of absorbed light of the reference dye fluorescein in 0.1M NaOH

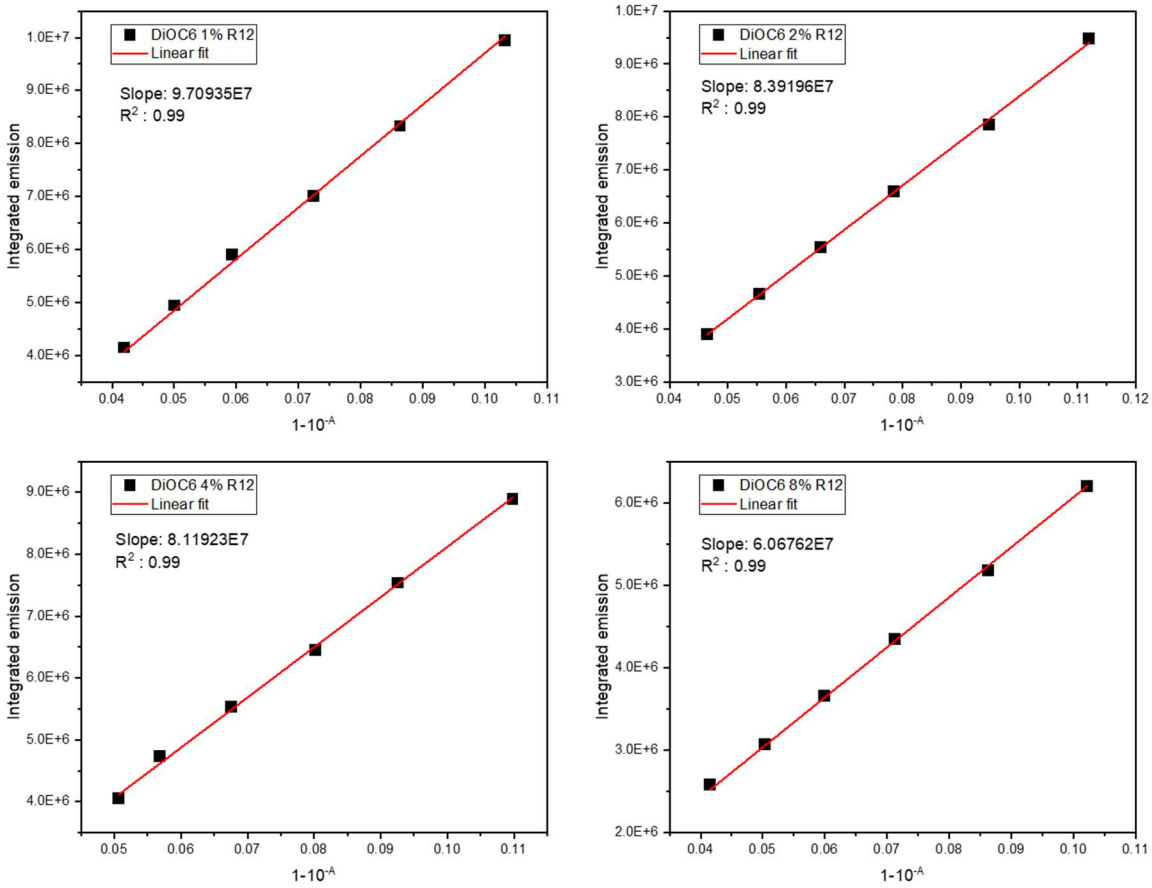

Figure S10 Linear fit of integrated emission as function of absorbed light of DiOC6:R12 nanoparticles 1-8mol%

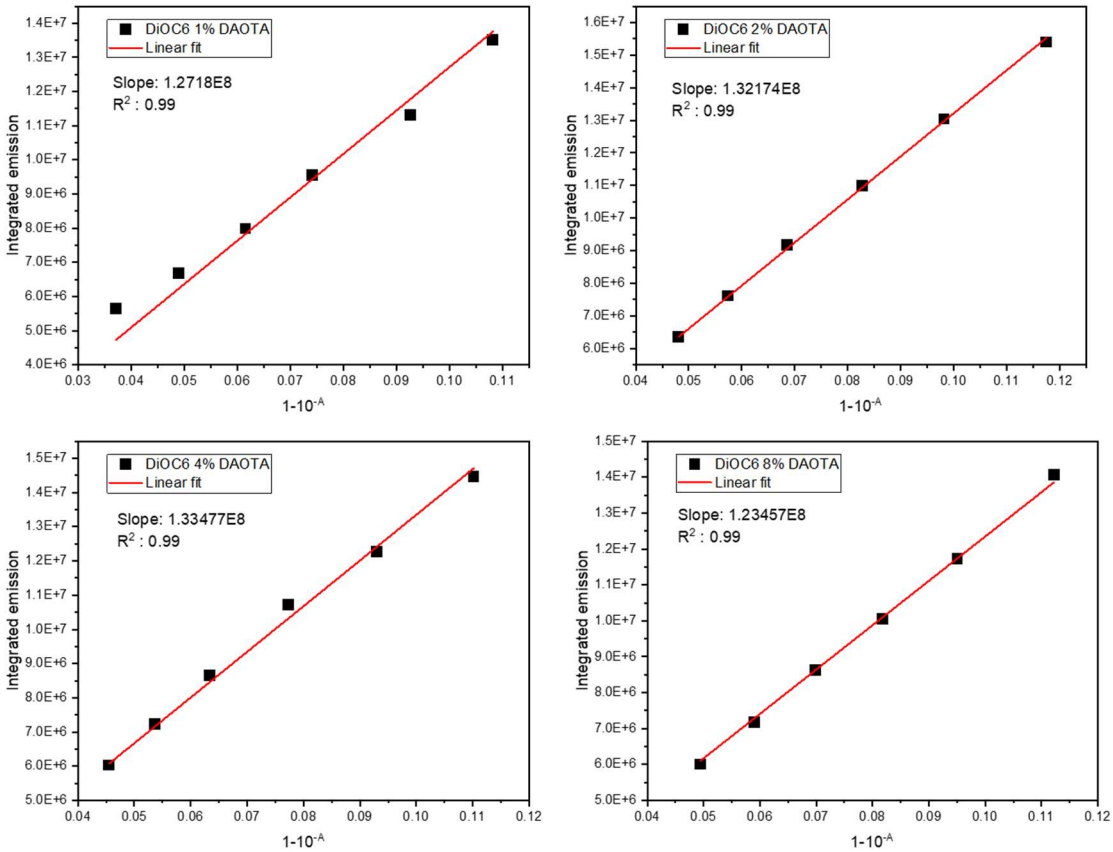

Figure S11 Linear fit of integrated emission as function of absorbed light of DiOC6:DAOTA NPs 1-8 mol%

Lifetime imaging microscopy

Single particle fluorescence lifetime microscopy images were recorded on a home-built confocal microscope setup described in detail previously.[9] We used a SuperK ExtremeEXB-6 supercontinuum laser (NKT Photonics) for excitation and an avalanche photodiode from Excelitas (SPCM-AQRH-14-TR) for fluorescence detection. An excitation wavelength of 485 nm was selected using the Super K SELECT wavelength selector from NKT Photonics and a repetition rate of 2.4 MHz was used. The excitation light was guided towards an oil immersion objective (Olympus UPlanSApo 100x, 1.4 NA) with a 30/70 beam splitter (XF122, Omega Optical). A LL01-485/20-25 band pass filter from Semrock was used for cleaning up the excitation light and a BLP01-561R-25 and LP02-488Re-25 from Semrock was used in the detection path. Dilute solutions of SMILES NPs were spin-coated on clean cover slips and imaged after drying.

The BLP01-561R-25 was used to mainly detect emission from the acceptor fluorophores (R12 or DAOTA), as the majority of the emission intensity is from these and not the donor DiOC6 after 560 nm.

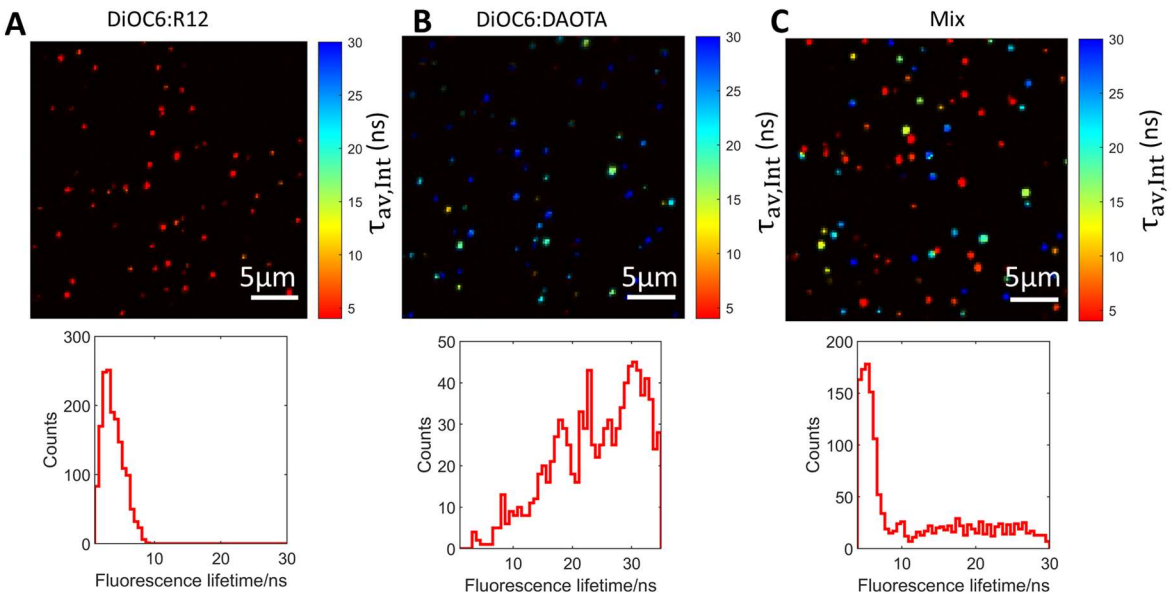

Figure S12 FLIM images of a) DiOC6 1% R12, b) DiOC6 4% DAOTA NPs, and c) a mixture of both with corresponding histograms of the lifetimes.

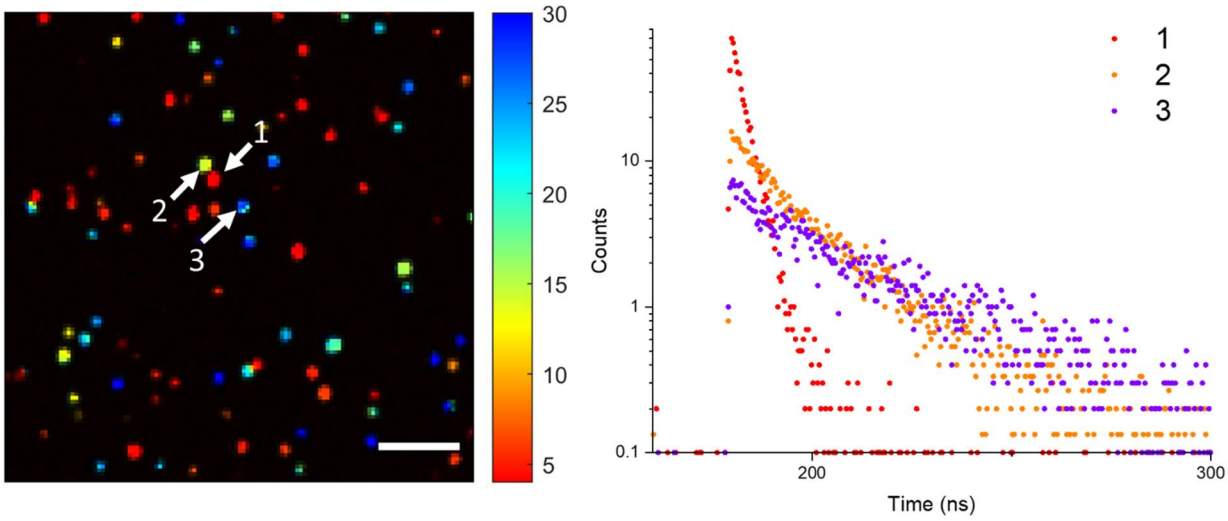

Figure S13 FLIM image of intensity average lifetime in ns and fluorescence decays of selected particles 1, 2 and, 3 indicated by the arrows. Scale bar 5 µm.

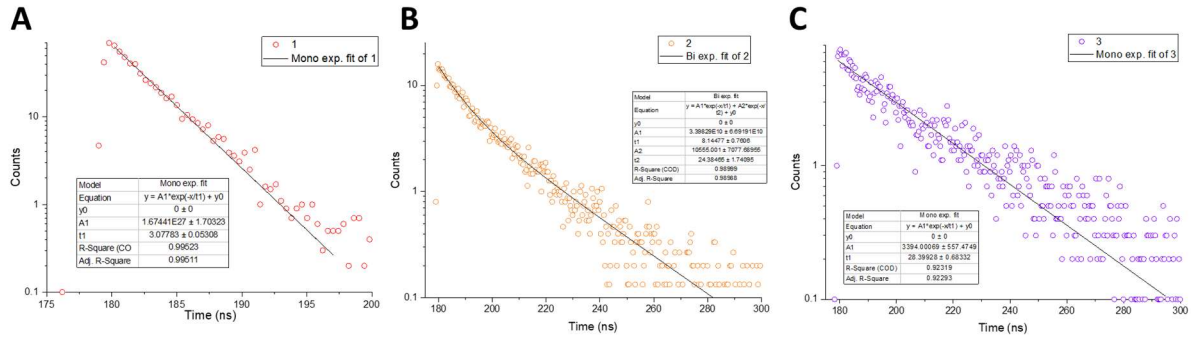

Figure S14 Exponential decay tail fits of the 3 extracted decays. A) Particle 1, mono-exponential fit τ = 3.1 ns, B) Particle 2, bi-exponential fit τ_1 = 8.1 ns and τ_2 = 24.4 ns and C) Particle 3, mono-exponential fit τ = 28.4 ns.

The time-gated images were prepared by applying two time gates either short (0-13ns) or long (58-193ns) where the total photon count of that gate is imaged (figure S14B and C). The pseudocoloured image (figure 7c) is achieved by overlaying the long-time gate image (blue) on the short-time gate (red).

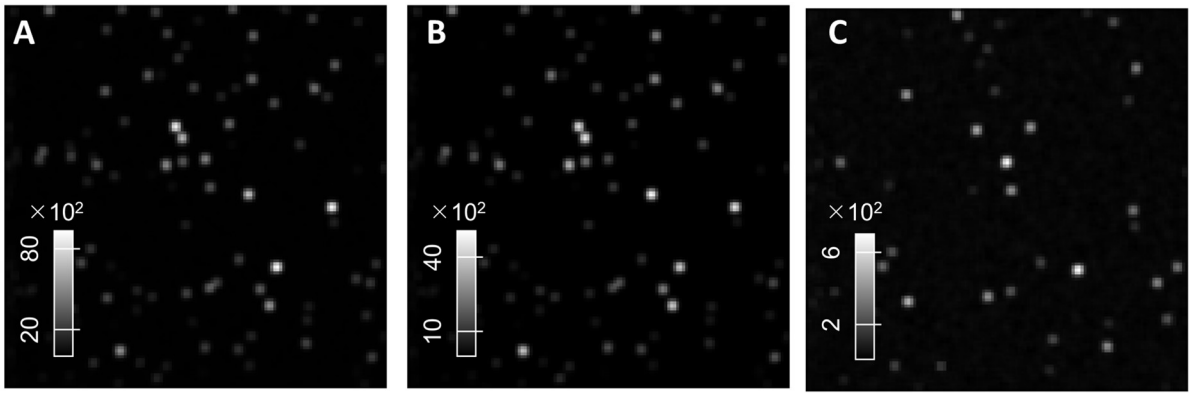

Figure S15 Intensity images of mixed doped NPs. A) Total intensity, B) short time-gate from 0-13 ns, and C) long time-gate from 58-193 ns after excitation.

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