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SUPPORTING INFORMATION

Spectroscopic differentiation between monomeric and aggregated forms of BODIPY dyes: Effect of 1,1-dichloroethane

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Materials and methods

All reagents and solvents were purchased from commercial sources (Sigma-Aldrich, Acros, Alfa, Aesar) and were used as received. For information on 1,1-DCE and 1,2-DCE samples, see Table S1.

Absorbance and fluorescence measurements were performed on Agilent 8453 UV-visible and Shimadzu RF-5301PC instruments, respectively, using 1 cm quartz cells with a resolution of 1 nm. Fluorescence measurements were carried out as follows: excitation and emission width slits were 3 nm and 3 nm; intensity – high or low; samples were excited either at 480 nm, and the obtained spectra were smoothed using manufacture provided software.

BODIPY dyes: all BODIPY dyes were either available from previous studies or prepared according to published procedures.[1]

Stock and solution preparations for spectroscopic measurements: 1.0 mM stock solutions of a dye was prepared by dissolving a weighted amount of the dye in the appropriate solvent, followed by sonication for 30-40 sec. All stock solutions were prepared fresh, and used within 24 hours. For the all measurements the aliquots of the stock solutions were diluted to the appropriate solvent, thoroughly mixed and allowed to equilibrate for several minutes prior to the spectra acquisition.

Structures of BODIPY dyes

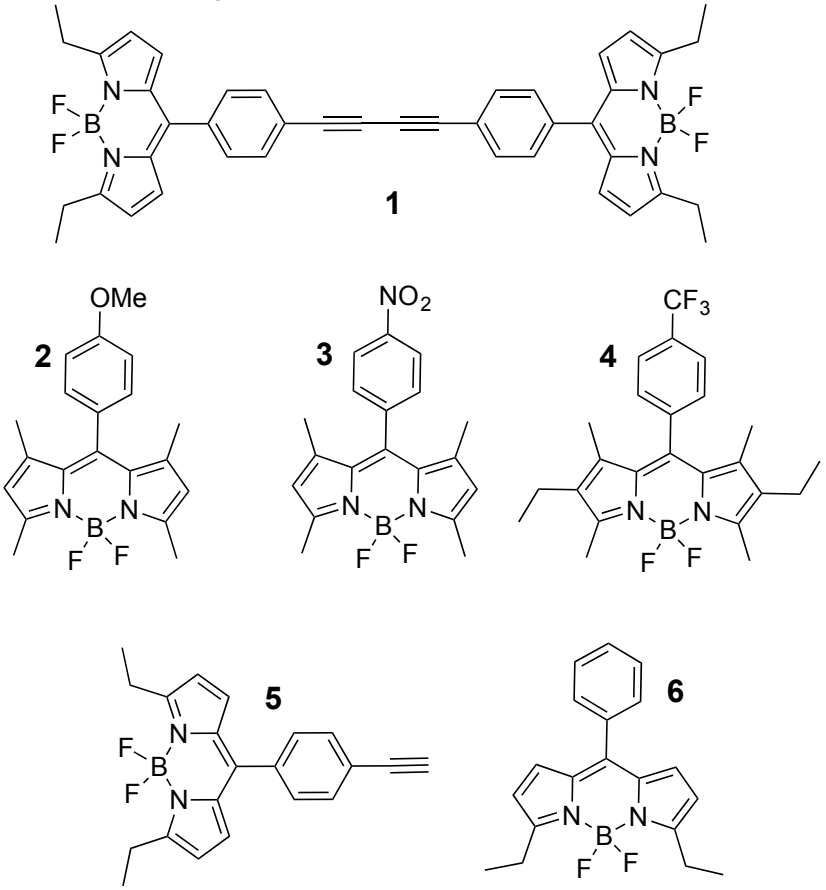


Chart 1. Structures of BODIPY dyes.

Anisotropy and lifetime measurements

Fluorescence lifetimes were measured on a FluoTime 300 fluorometer (PicoQuant, Inc.) using 500±10 nm light from a Fianium supercontinuum laser. The fluorometer was equipped with an ultrafast microchannel plate detector (MCP) from Hamamatsu, Inc. The fluorescence lifetimes were measured under magic angle conditions using the appropriate long pass filter and data were analyzed with FluoFit4 program from PicoQuant, Inc (Germany) using multi-exponential fitting model:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (1)$$

where, α_i is the fractional amplitude of the intensity decay of the i^{th} component at time t and τ_i is the lifetime of the i^{th} component. The amplitude and intensity weighted average lifetimes ($\langle\tau\rangle_{amp}$, and $\langle\tau\rangle_{int}$) were calculated using the following equations:

$$\langle\tau\rangle_{amp} = \frac{\sum_i \alpha_i\tau_i}{\sum_i \alpha_i} \quad (2)$$

$$\langle\tau\rangle_{int} = \sum_i f_i\tau_i \quad where \quad f_i = \frac{\alpha_i\tau_i}{\sum_i \alpha_i\tau_i} \quad (3)$$

and f_i represents fractional intensities for each fluorescence lifetime component.

The (normalized or reduced) χ^2 is the optimization parameter for least squares fitting analysis defined as:

$$\chi^2 = \frac{1}{n-p}\sum_{i=1}^{n}\left[\frac{I_i - I(t_i)}{\sigma_i}\right]^2 \quad (4)$$

n is the number of data points, p is the number of freely varying parameters, σ_i is the standard deviation, I_i is the measured intensity at time t_i, and $I(t_i)$ is the fitted theoretical value for the fitted intensity decay function at time t_i.[2]

Time resolved anisotropy measurements were carried out with the same FT300 fluorometer (Picoquant GmbH, Germany) using a 500 ± 10 nm excitation diode laser (PicoQuant) operated at 10 MHz repetition rate. The emission was observed at the peak emission wavelength supported with the appropriate long pass filter to eliminate excitation light. The resolution was kept at 4 ps per channel and the pulse width was less than 100 ps. Fluorescence intensity decays were collected while orienting the emission polarizer in vertical and horizontal positions respective to the vertically oriented excitation polarizer for measuring anisotropy decays. The vertical (parallel) and horizontal (perpendicular) intensity decays were used to calculate the time dependent anisotropy according to:

$$r(t) = \frac{I_{Parallel}(t) - I_{Perpandicular}(t)}{I_{Parallel}(t) + 2I_{Perpandicular}(t)} \qquad (5)$$

The obtained anisotropy decay was analyzed using Fluofit 4.0 program provided by Picoquant GmbH, Germany, and fitted using formula:

$$r(t) = \sum r_i \, e^{-t/\phi_i} \qquad (6)$$

where, r(t) is the time-dependent total anisotropy decay, r_i is the fractional anisotropy amplitude associated with ith component, and φi is the rotational correlation time.

For the tables, the abbreviations are as follows:

τ_1, τ_2, τ_3 – lifetime components
α_1, α_2 and α_3 – respective amplitudes
$\langle\tau\rangle_{amp}$ – amplitude weighted average lifetime
$\langle\tau\rangle_{int}$ – intensity weighted average lifetime
χ^2 – optimization parameter for least squares fitting analysis
r_o – anisotropy at time zero, r_{inf} represents population with fraction of anisotropy with very long correlation time compared to lifetime (φ>> τ)
Φ – rotational correlation time
$r_{o\ fitted}$ – recovered anisotropy at zero time from fit
$r_{o\ app}$ – measured anisotropy at zero time
SS r – steady state anisotropy

Relative quantum yields for BODIPY dyes were calculated according to the following equation:

$$\Phi_x = \Phi_s \, (A_s/A_x)(F_x/F_s) \, (n_x/n_s)^2$$

where Φ_s – quantum yield of the standard; A – the absorbance (within 0.01 – 0.08 au range), F – the area under the emission curve, n – refractive index, the subscripts *s* and *x* are the standard and the unknown, respectively. For the quantum yield measurements, the dyes were excited at 480 nm, excitation and emission slits were both set at 3 nm. Rhodamine 6G was used as a the standard (Φ_s = 0.95 in EtOH).[3]

Conditions for GC-VUV analysis:

GC: Rtx-Q-BOND column (30 m, 0.32 mm I.D., 10 µm d_f); injection: 250 °C, 0.2 µL splitless; oven: 35 °C (1 min), 20 °C/min to 250 °C (30 min); carrier gas: He @26 cm/s.

VUV: interface and flow cell@ 275 °C; make-up gas@ 0.15 psi; wavelength@ 125-160 nm

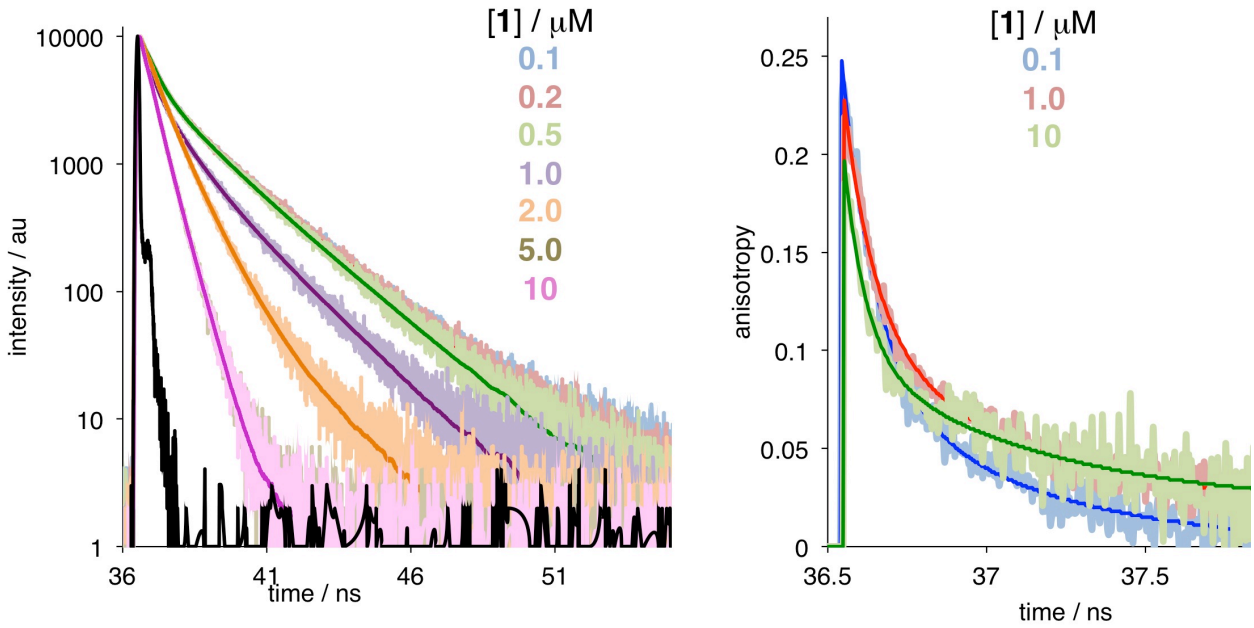


Figure S1. Concentration-dependent fluorescent lifetimes and anisotropy of dye **1** in 1,2-DCE (ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details).

Left: fluorescent lifetime measurements; solid darker lines – calculated fits; ref – black curve. Right: anisotropy measurements.

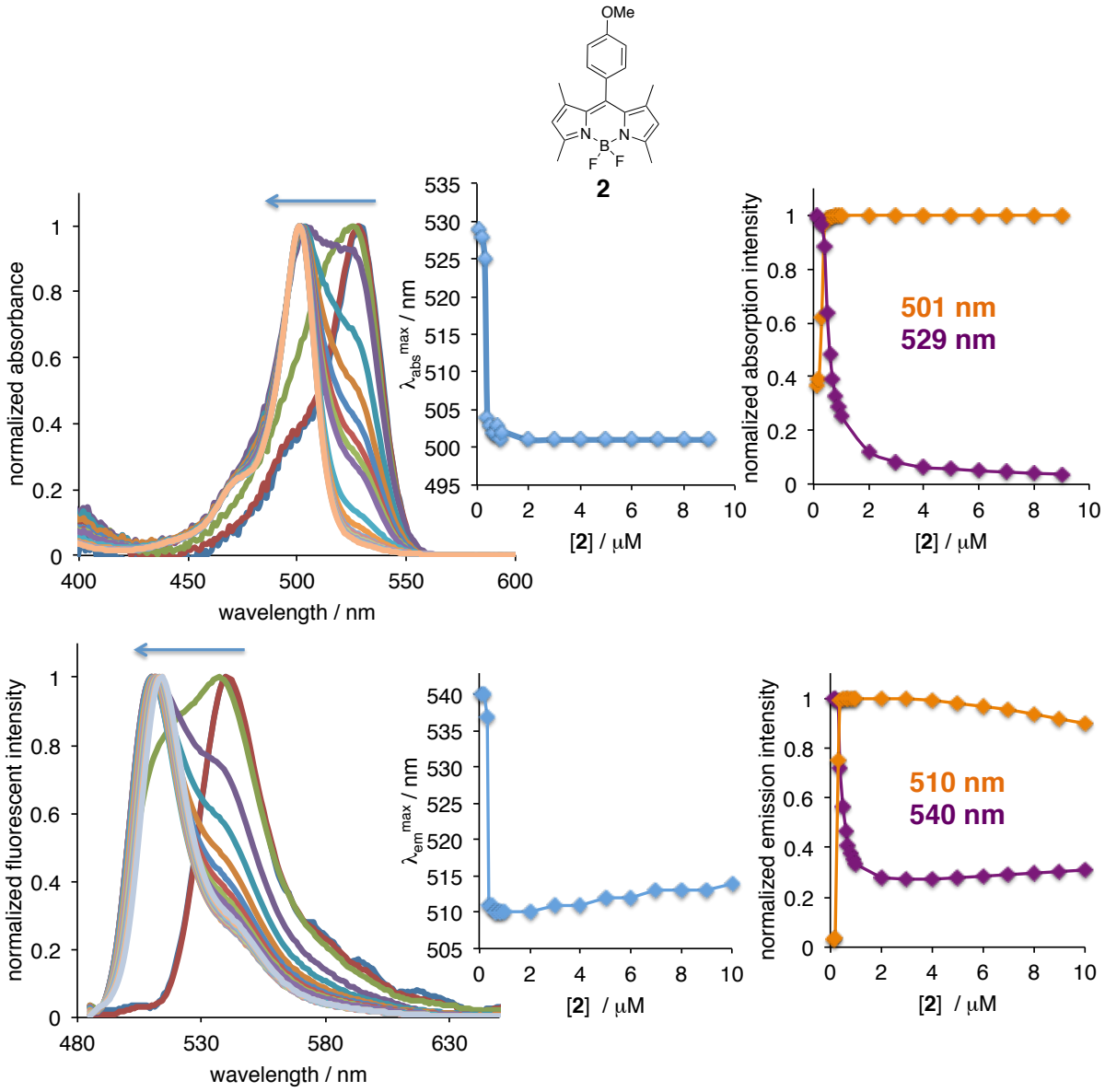


Figure S2 A: Absorption (top) and emission (bottom) spectra of BODIPY dye **2** in 1,2-DCE. The arrow above the spectra shows the direction of the shift upon increasing the concentration of the dye. Insets: changes in the absorption and emission maxima as a function of concentration, and changes in the absorption and emission intensities at the maxima of the monomer and aggregate as a function of dye concentration.

λ ex = 480 nm; dye **2** stock 1.0 mM prepared in 1,2-DCE.
1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

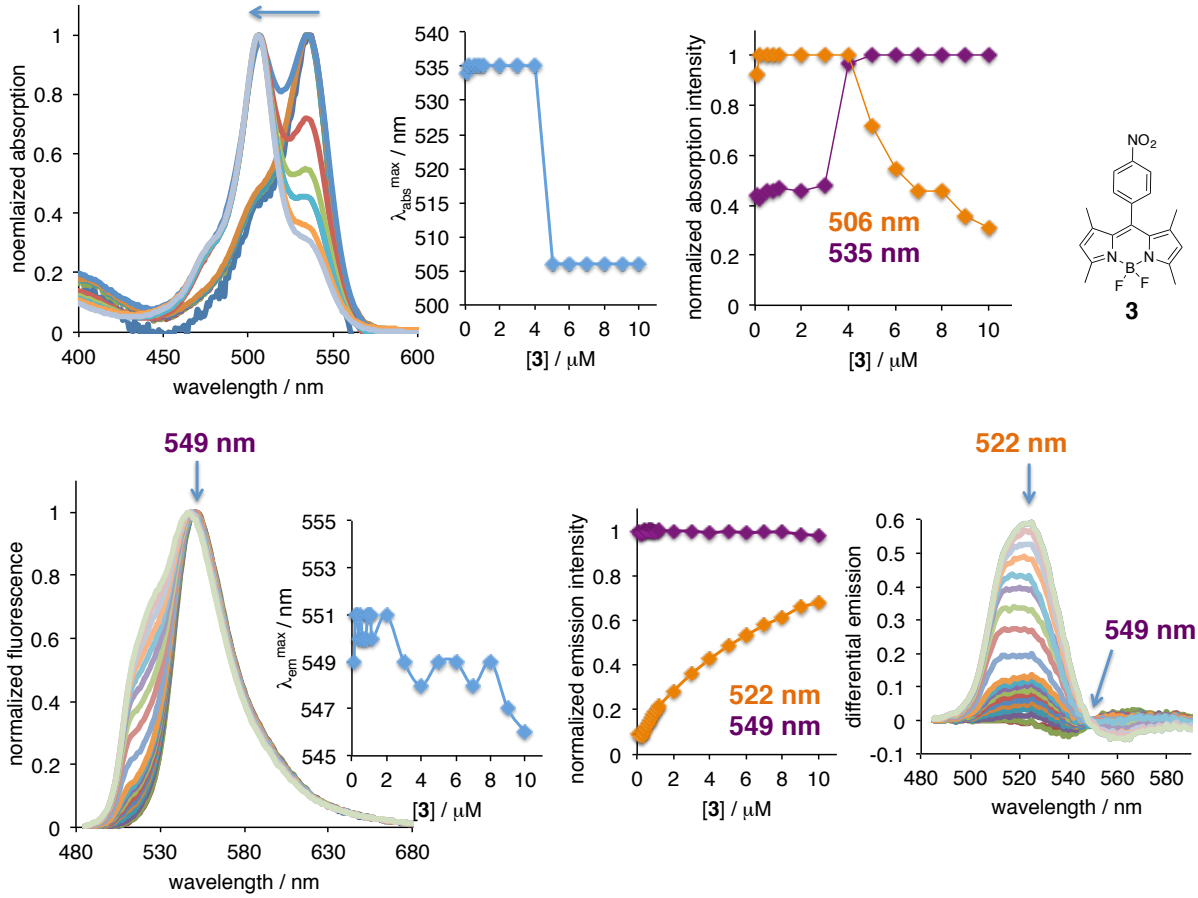


Figure S2 B: Absorption (top) and emission (bottom) spectra of BODIPY dye **3** in 1,2-DCE.

The arrow above the absorption spectra shows the direction of the shift upon increasing the concentration of the dye. Insets: changes in the absorption and emission maxima as a function of concentration, and changes in the absorption and emission intensities at the maxima of the monomer and aggregate as a function of dye concentration.

Differential emission spectra (bottom right inset) show the increase in intensity of the aggregated form of the dye at emitting at 522 nm (spectra were obtained as follows: $I_F^{10} - I_F^n$, where I_F^{10} is the normalized emission spectra of [**3**] = 10μM, and I_F^n are normalized emission spectra of [**3**] = n μM, with n = 0.1, 0.2, … 8.0, 9.0 μM).

λ ex = 480 nm; dye **3** stock 1.0 mM prepared in 1,2-DCE;
1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

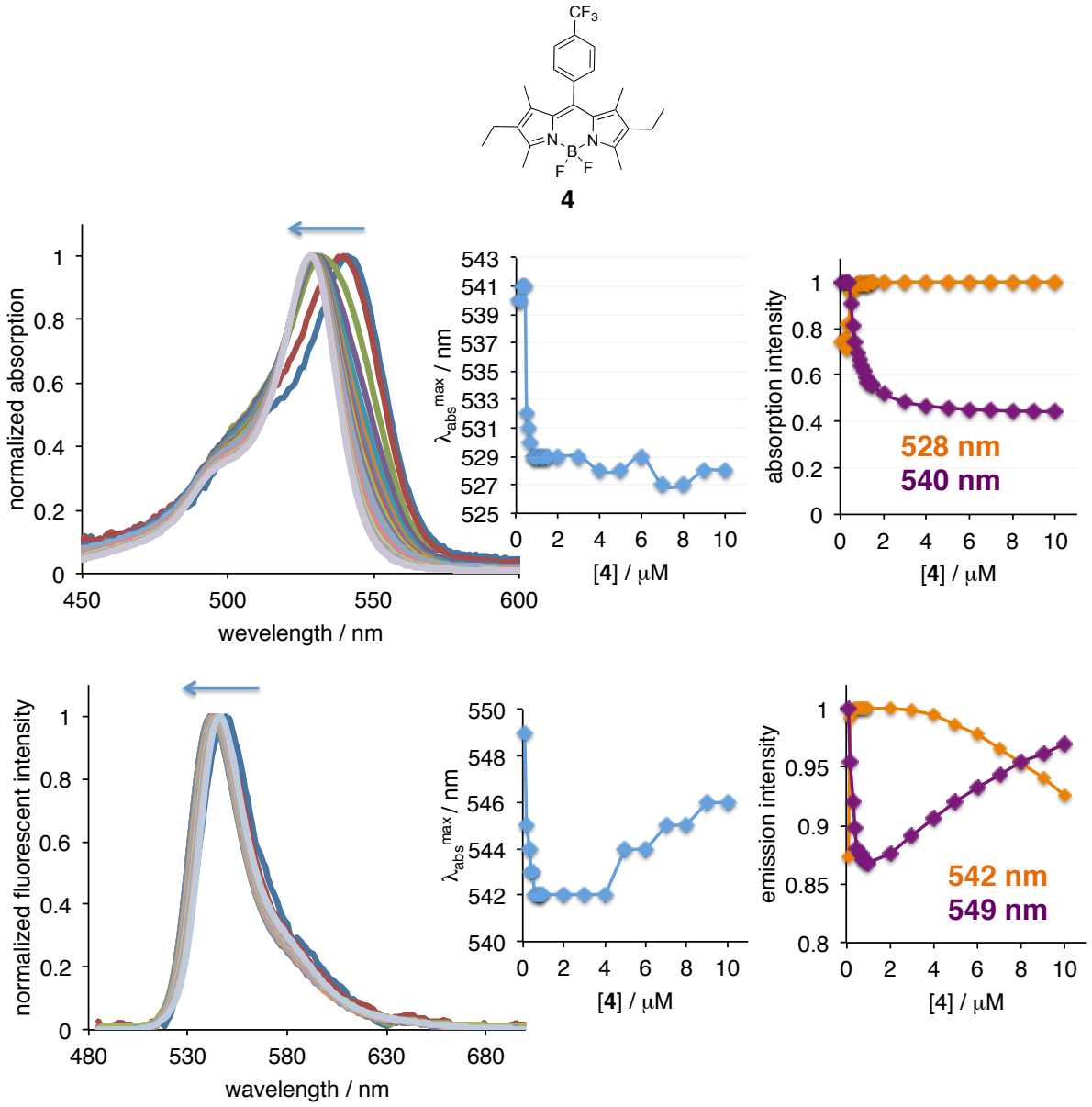


Figure S2 C: Absorption (top) and emission (bottom) spectra of BODIPY dye **4** in 1,2-DCE. The arrow above the spectra shows the direction of the shift upon increasing the concentration of the dye. Insets: changes in the absorption and emission maxima as a function of concentration, and changes in the absorption and emission intensities at the maxima of the monomer and aggregate as a function of dye concentration.

λ ex = 480 nm; dye **4** stock 1.0 mM prepared in 1,2-DCE;
1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

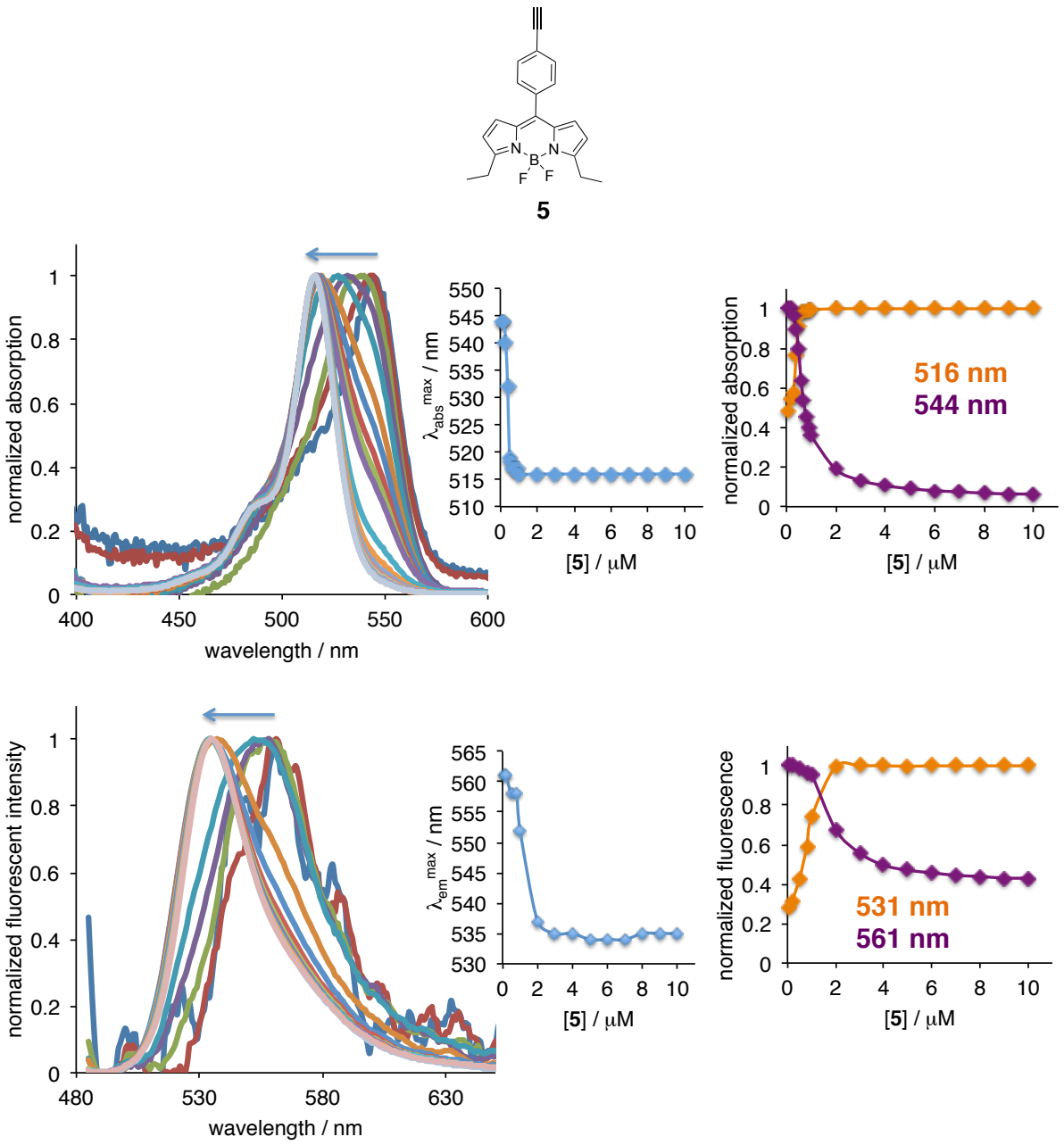


Figure S2 D: Absorption (top) and emission (bottom) spectra of BODIPY dye **5** in 1,2-DCE. The arrow above the spectra shows the direction of the shift upon increasing the concentration of the dye. Insets: changes in the absorption and emission maxima as a function of concentration, and changes in the absorption and emission intensities at the maxima of the monomer and aggregate as a function of dye concentration.

λ ex = 480 nm; dye **5** stock 1.0 mM prepared in 1,2-DCE
1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

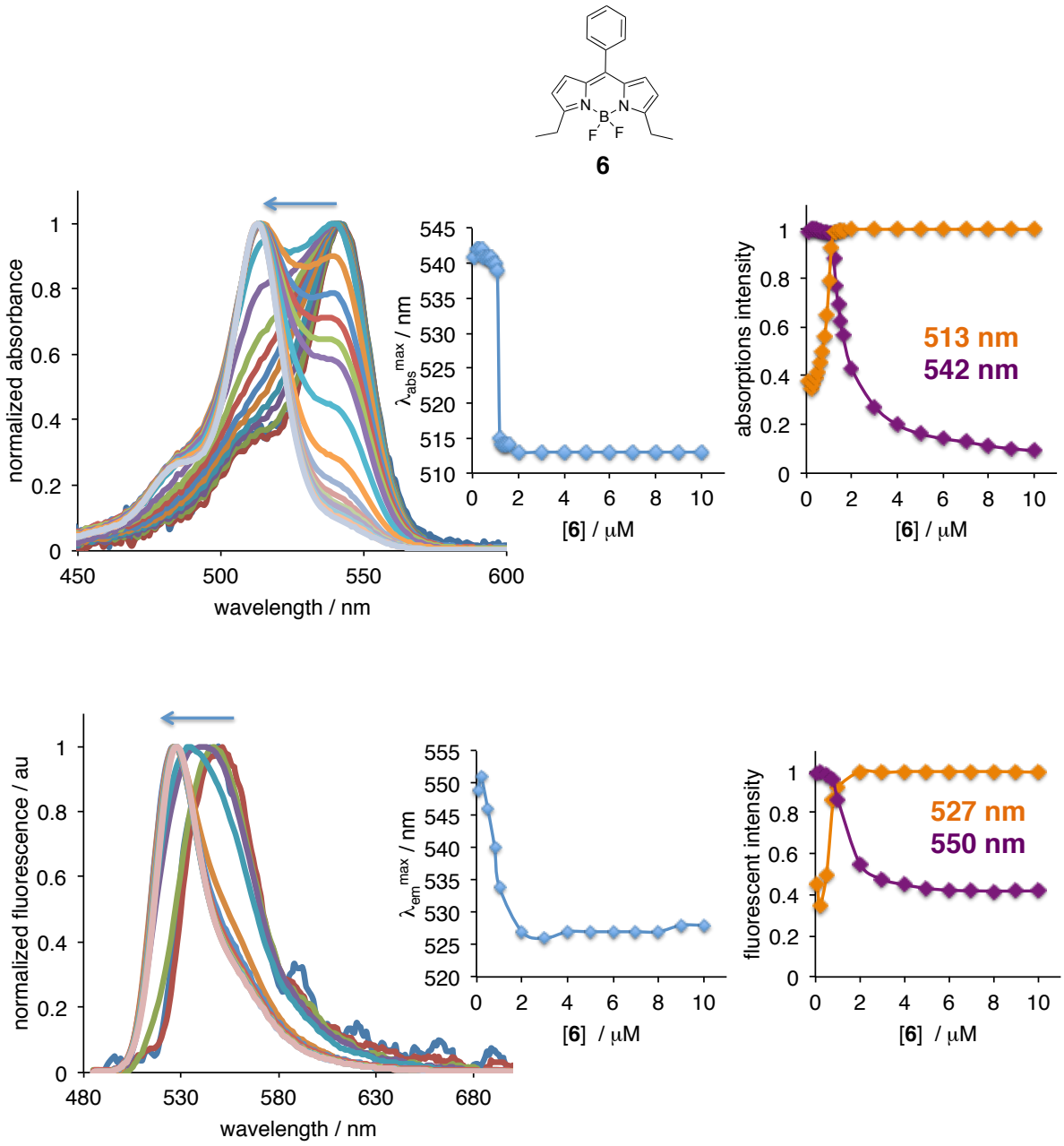
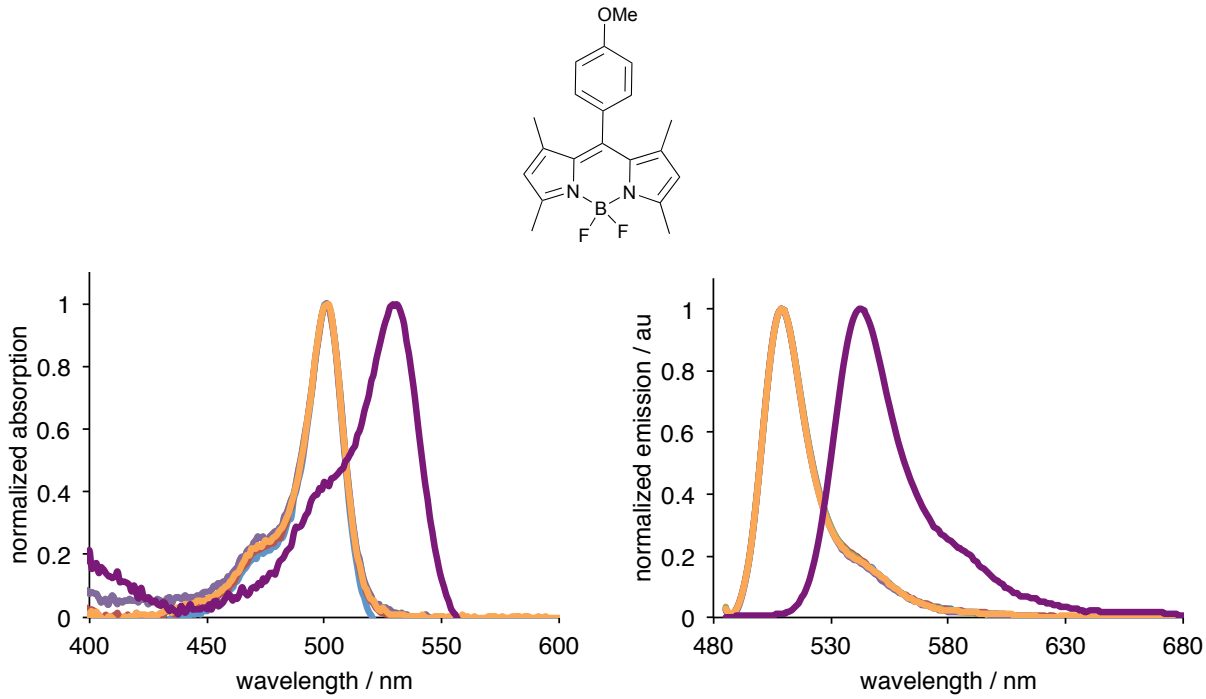


Figure S2 E: Absorption (top) and emission (bottom) spectra of BODIPY dye **6** in 1,2-DCE. The arrow above the spectra shows the direction of the shift upon increasing the concentration of the dye. Insets: changes in the absorption and emission maxima as a function of concentration; and changes in the absorption and emission intensities at the maxima of the monomer and the aggregate as the function of dye's concentration.

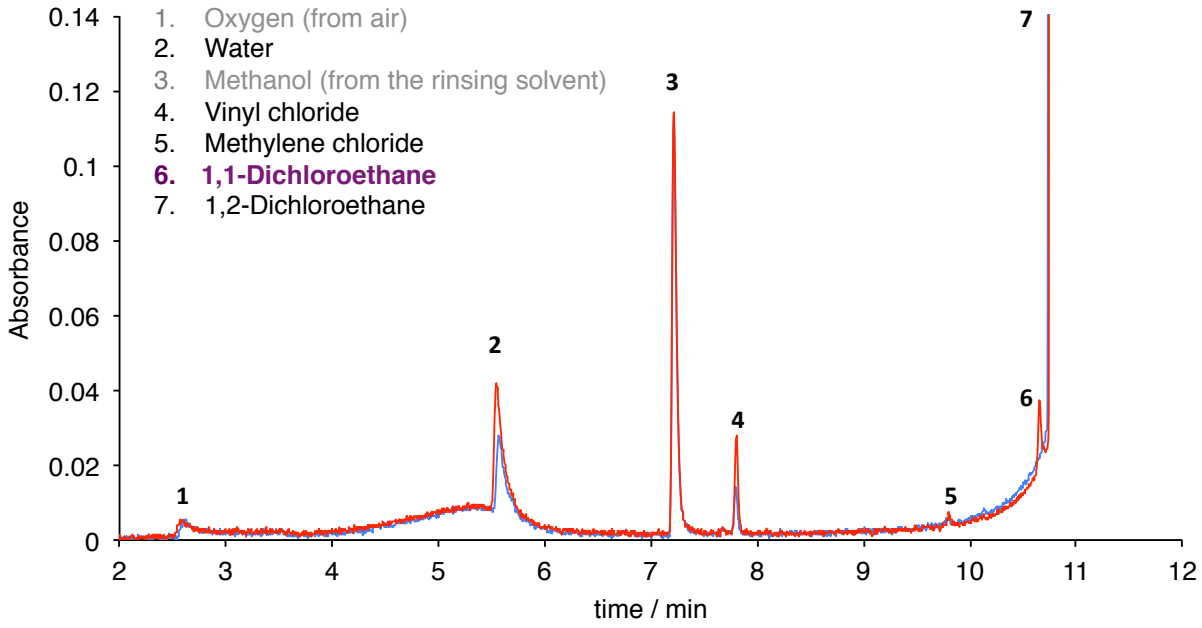
λex = 480 nm; dye **6** stock 1.0 mM prepared in 1,2-DCE;
1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.



1,2-DCE[a] vendor/description	λ_{ab}^{max} / nm	FWHM / nm[b]	λ_{em}^{max} / nm	FWHM / nm[c]
ACROS / extra dry	501	19	509	22
ACROS / HPLC	501	19	509	22
ACROS / ACS-spectrograde	501	19	509	22
ACROS / for spectroscopy	***529***	***31***	***543***	***31***
ALDRICH / anhydrous	501	19	509	22
ALDRICH / CHROMASOL for HPLC	501	19	509	22

Figure S3. Spectral characteristics of BODIPY **2** in 1,2-DCE from various vendors.
absorption spectra – left; emission spectra – right
[a] – see Table S1 for additional details.
[b] – FWHM: full width at half maximum calculated from the absorption spectra
[c] – FWHM: full width at half maximum calculated from the emission spectra

[**2**] = 1.0μM
emission spectra: λex = 480 nm



impurity	1,2-DCE / ACROS,[a] μg/ml	1,2-DCE / ACROS,[b] μg/ml
water (2)	192.0 ± 3.8	80.4 ± 2.9
vinyl chloride (4)	54.9 ± 1.8	19.10 ± 0.92
CH_2Cl_2 (5)	7.35 ± 0.22	6.04 ± 0.42
1,1-DCE (6)	56.57 ± 0.78	–

Figure S4. GC-VUV chromatographs of 1,2-DCE samples and quantitative analysis of impurities.

[a] – 1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.
[b] – 1,2-DCE is ACROS/ACS-spectrograde, catalogue #406835000; see Table S1 for additional details.

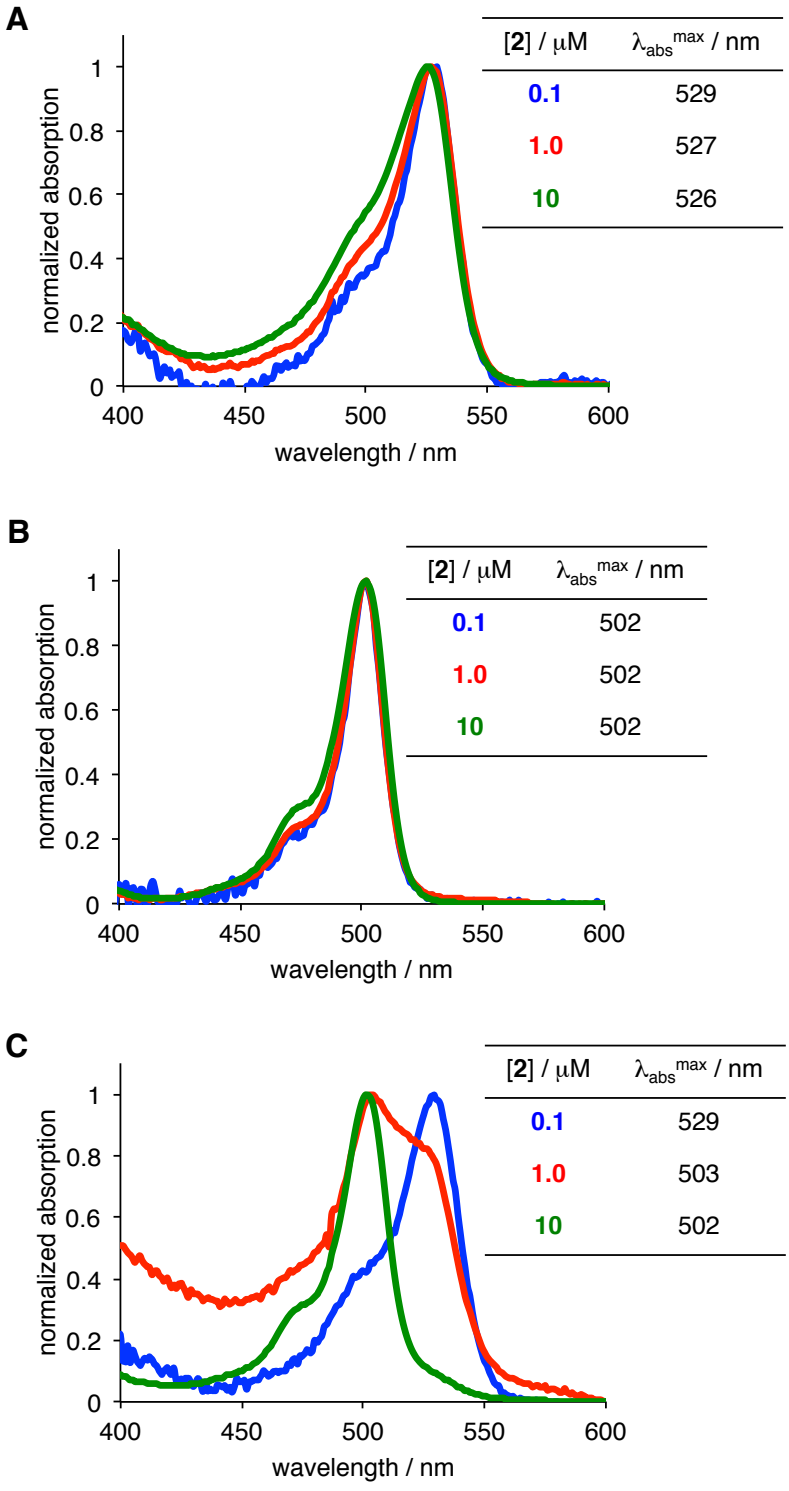


Figure S5. Normalized absorption spectra of dye **2** as a function of concentration in 1,1-DCE and 1,2-DCE.
A: **2** in 1,1-DCE;
B: **2** in 1,2-DCE-ACROS/ACS-spectrograde;
C: **2** in 1,2-DCE-ACROS/for spectroscopy.
See Table S1 for additional details on the solvents.

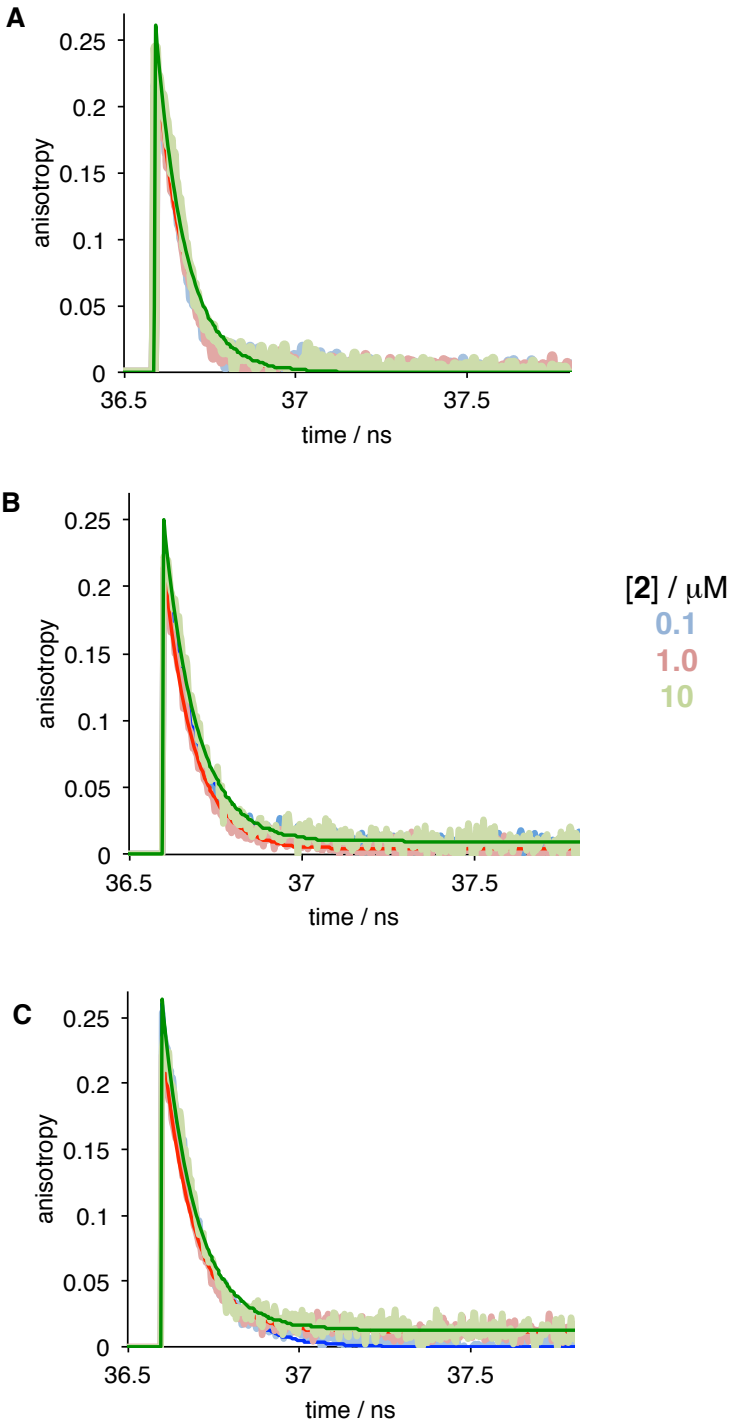


Figure S6. Anisotropy of dye **2** as a function of concentration 1,1-DCE and 1,2-DCE. Solid darker lines – calculated fits.

A: **2** in 1,1-DCE;
B: **2** in 1,2-DCE-ACROS/ACS-spectrograde;
C: **2** in 1,2-DCE-ACROS/for spectroscopy.
See Table S1 for additional details.

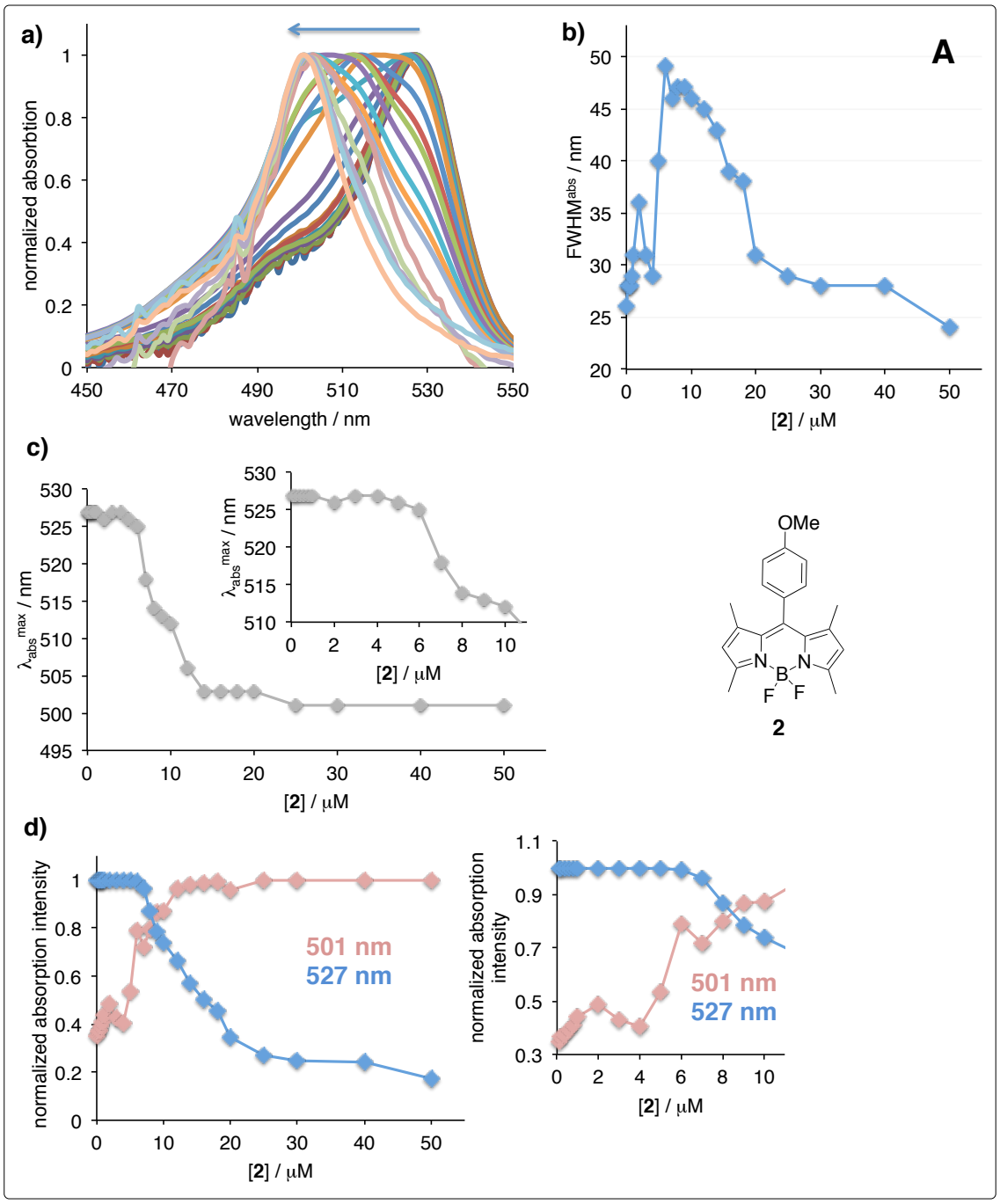


Figure S7A. Absorption spectra of dye **2** in 1,1-DCE.

a) Normalized absorption spectra (the arrow above the spectra shows the direction of the shift upon increasing the concentration of the dye); b) changes of the FWHM as a function of dye's concentration; c) changes in the absorption maxima as a function of dye's concentration; d) changes in the absorption intensities at the maxima of the monomer and aggregate as a function of dye concentration.

Absorptions spectra were acquired in 1 cm cell for [**2**] = 0.1 – 18 μM, and 0.01 cm demountable cell for [**2**] = 20 – 50 μM

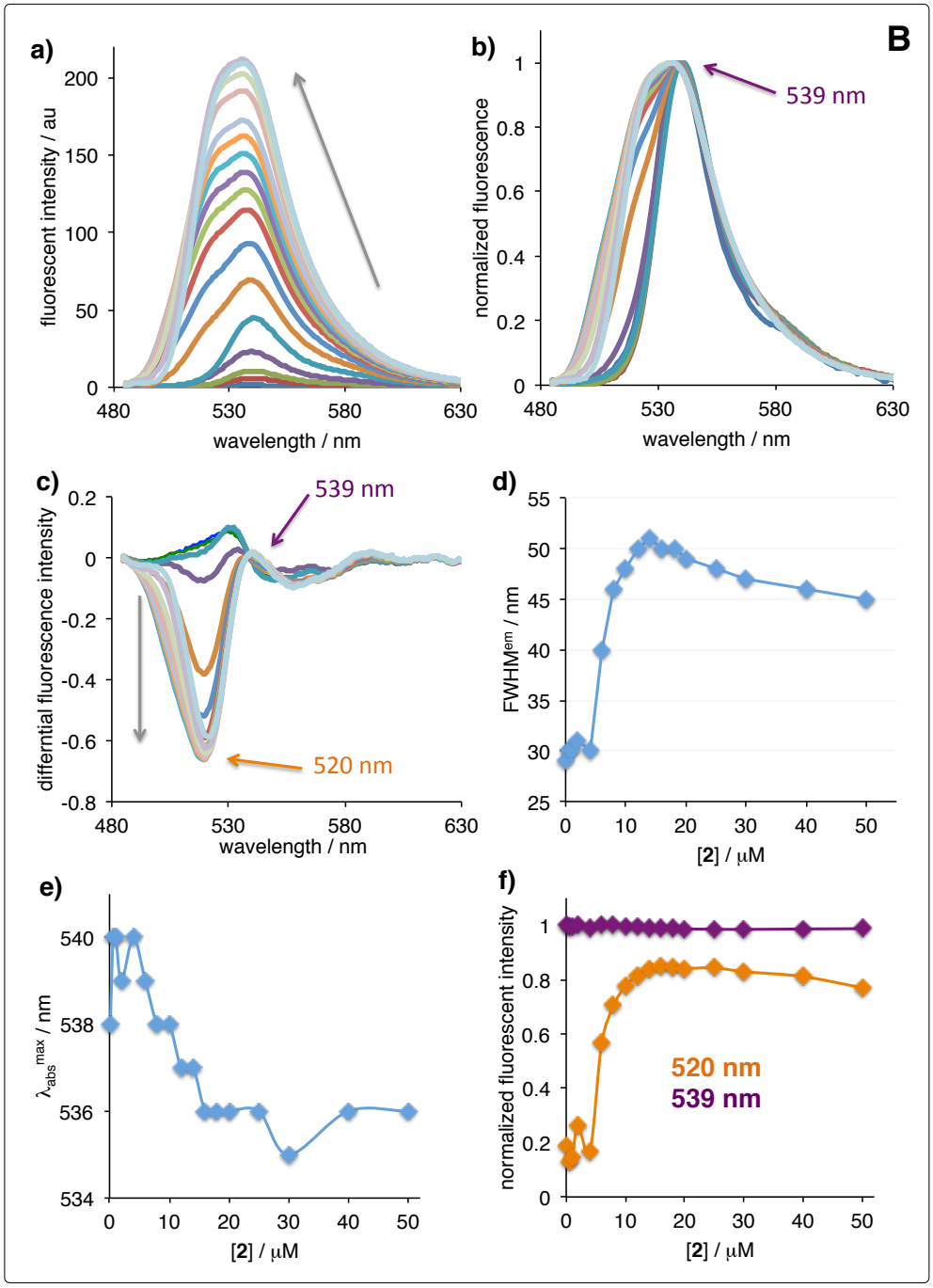


Figure S7B. Emission spectra of dye **2** in 1,1-DCE.

a) concentration dependent emission spectra (the arrow shows the direction of the shift upon increasing the concentration of the dye); **b**) normalized emission spectra; **c**) Differential emission spectra (spectra were obtained as follows: $I_F^n - I_F^{50}$, where I_F^{50} is the normalized emission spectra of [**2**] = 50 μM, and I_F^n is the normalized emission spectra of [**2**] = n μM, with n = 0.1, 0.2, … 8.0, 9.0, etc μM); **d**) changes of FWHM as a function of dye's concentration; **e**) changes of emission maxima as a function of dye's concentration; **f**) changes in the normalized emission intensities at the maxima of the monomer and aggregate as a function of dye's concentration.

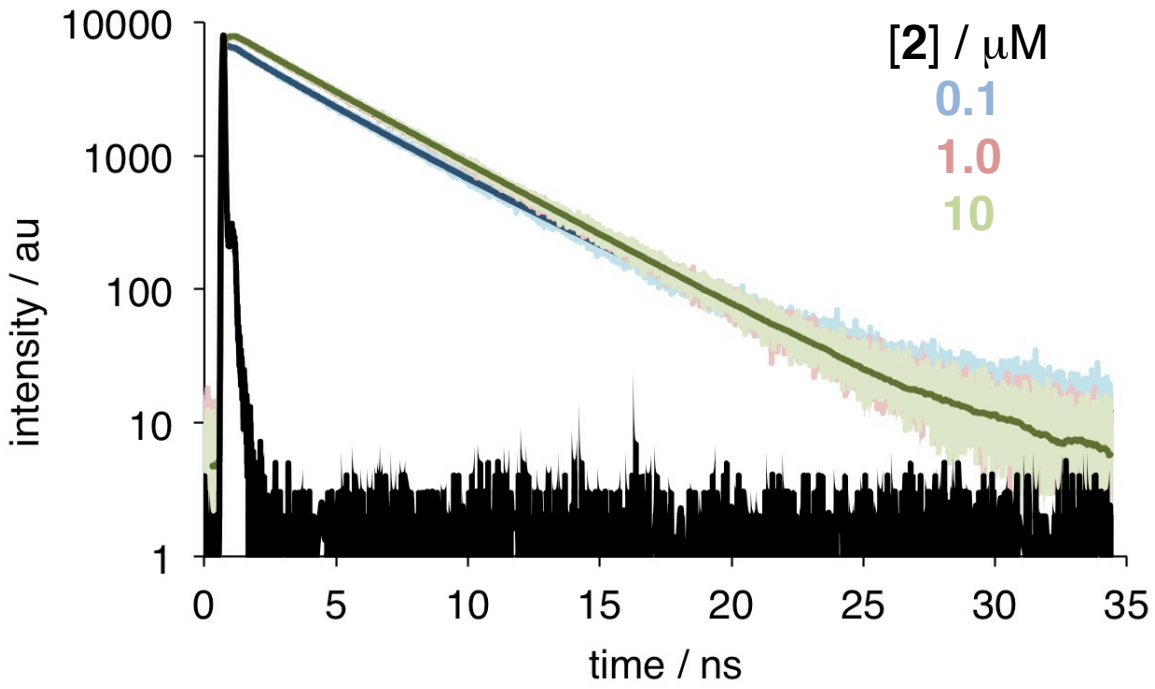


Figure S8. Concentration-dependent fluorescent lifetimes of dye **2** in 1,1-DCE.

solid darker lines – calculated fits; reference – black curve.

Table S1. List of 1,1-DCE and 1,2-DCE samples used in this work.

	vendor	order number	lot	purity / %	description	comment
1,1-DCE						
	TCI	D0363	OPP3G	>95		stabilized with nitromethane
	Aldrich	36967	SZBD242XV	99.1		
1,2-DCE						
	Aldrich	284505	SHBG5649V	99.8	anhydrous	
	Aldrich	34872	MKBV45654	>99.8	chromasolv for HPLC	
	ACROS	326841000	1351608	99.8	extra dry	N_2 flushed
	ACROS	433580010	A0329104	99.8	HPLC	N_2 flushed
	ACROS[a]	***167765000***	***A0324426***	***99+***	***for spectroscopy***	***N_2 flushed***
	ACROS[b]	406835000		99+	ACS spectrograde	

[a] – 1,2-DCE used in all initial studies as well as for the GC-VUV analysis
[b] – 1,2-DCE used for the GC-VUV analysis

Table S2. Concentration-dependent fluorescent lifetimes for dye **1** in 1,2-DCE.[a]

[**1**] / μM	τ_1 / ns	τ_2 / ns	τ_3 / ns	α_1	α_2	α_3	$\langle\tau\rangle_{amp}$ / ns	$\langle\tau\rangle_{int}$ / ns	χ^2
0.1	0.40	1.30	2.50	0.59	0.17	0.24	1.05	1.75	0.88
0.2	0.40	1.16	2.40	0.58	0.16	0.27	1.04	1.74	0.90
0.5	0.38	1.39	2.40	0.58	0.20	0.22	1.01	1.68	0.91
1.0	0.26	0.87	1.90	0.55	0.30	0.15	0.70	1.22	0.90
2.0	0.28	0.70	1.44	0.22	0.69	0.09	0.67	0.81	0.87
5.0	0.34	0.58	–	0.67	0.33	–	0.41	0.45	0.90
10	0.27	0.53	–	0.49	0.51	–	0.40	0.44	0.99

[a] – 1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

Table S3. Concentration-dependent anisotropy measurements for dye **1** in 1,2-DCE.[a]

[1] / μM	r_o	r_1	r_2	r_{inf}	Φ_1 / ps	Φ_2 / ps	SSr	χ^2
0.1	0.243	0.150	0.093	–	93 ± 6	541 ± 35	0.043	0.98
0.2	0.237	0.151	0.088	–	93 ± 6	541 ± 35	0.041	0.99
0.5	0.258	0.147	0.090	0.021	93 ± 6	541 ± 35	0.064	1.05
1.0	0.228	0.114	0.095	0.018	93 ± 6	541 ± 35	0.071	1.14
2.0	0.200	0.108	0.076	0.016	93 ± 6	541 ± 35	0.072	1.03
5.0	0.194	0.104	0.065	0.025	93 ± 6	541 ± 35	0.088	1.21
10	0.188	0.098	0.060	0.026	93 ± 6	541 ± 35	0.089	1.17

[a] – 1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.

Table S4. Concentration-dependent anisotropy measurements for dye **2** in 1,1-DCE and 1,2-DCE.

solvent	[2] / μM	$r_{o\ fitted}$	$r_{o\ app}$	r_{inf}	Φ / ps	SSr	χ^2
1,2-DCE[a] ACROS	0.1	0.260	0.252	–	102 ± 2	0.006	1.6
	1.0	0.234	0.222	0.011	91 ± 3	0.017	1.2
	10	0.263	0.224	0.013	95 ± 3	0.019	1.4
1,2-DCE[b] ACS	0.1	0.228	0.208	0.009	92 ± 3	0.015	1.5
	1.0	0.217	0.213	0.004	86 ± 3	0.010	1.1
	10	0.250	0.222	0.009	94 ± 4	0.015	1.1
1,1-DCE	0.1	0.243	0.215	–	81 ± 3	0.003	1.4
	1.0	0.241	0.206	–	81 ± 3	0.002	1.5
	10	0.260	0.242	–	85 ± 3	0.005	1.5

[a] – 1,2-DCE is ACROS/for spectroscopy, catalogue #167765000; see Table S1 for additional details.
[b] – 1,2-DCE is ACROS/ACS-spectrograde, catalogue #1406835000; see Table S1 for additional details.

Table S5. Concentration dependent fluorescent lifetimes of dye **2** in 1,1-DCE.[a]

[2] / μM	τ_1 / ns	τ_2 / ns	τ_3 / ns	α_1	α_2	α_3	$\langle\tau\rangle_{amp}$ / ns	$\langle\tau\rangle_{int}$ / ns	χ^2
0.1	0.34	3.32	5.2	0.13	0.59	0.28	3.45	4.06	0.98
1.0	2.07	4.08	–	0.10	0.90	–	3.88	3.98	0.98
10	1.62	4.06	–	0.05	0.95	–	3.94	4.06	1.00

[a] – 1.0 mM stock of dye **2** was prepared in 1,1-DCE and subsequently diluted into 1,1-DCE to the desired concentration.

Table S6. Spectroscopic characterization of BODIPY dyes in 1,1-DCE.

DYE [a]	λ_{ab}^{max} / nm	λ_{em}^{max} / nm	τ / ns (χ^2) [b]	QY [c]
1	521	546	0.59 (0.82)	0.03
2	528	546	4.15 (0.99)	0.85
3	535	554	3.55 (0.96)	0.60
4	528	544	4.49 (0.98)	0.87
5	516	536	1.03 (0.85)	0.05
6	512	525	2.03 (0.92)	0.72

[a] – [dye] = 0.2 μM; the stock solution for all dyes were prepared in 1,1-DCE at 1.0 mM, and subsequently diluted into 1,1-DCE.

[b] – $\langle\tau\rangle_{int}$ – intensity weighted average lifetime; χ^2 – optimization parameter for least squares fitting analysis

[c] – QY – quantum yield; rhodamine 6G was used as a reference (Φ = 0.95 in EtOH).[3]

References

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