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SUPPORTING INFORMATION

INTERACTION OF BODIPY DYES WITH BOVINE SERUM ALBUMIN: A CASE STUDY ON THE AGGREGATION OF A CLICK-BODIPY DYE

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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. NMR spectra were recorded on a Varian (300 MHz) or Bruker (400 MHz) spectrometers. ^{1}H NMR chemical shifts are reported in ppm (δ) downfield from residual $CDCl_3$.

Absorbance and fluorescence measurements were performed on Agilent 8453 UV-visible instrument and Shimadzu RF-5301PC, respectively, using 1 cm quartz cells with a resolution of 1 nm at room temperature (20 ^{o}C). Fluorescence measurements were carried out as follows: excitation and emission width slits were 3 mm and 3 mm; intensity – high or low; samples were excited either at 475 nm or the absorption maximum, and the obtained spectra were smoothed using manufacture provided software.

Milli-Q-system was used to purify water that was utilized throughout this work. Stock solutions of the dyes for spectroscopic measurements were prepared in EtOH (0.10 mM) and subsequently diluted into the aqueous buffer. Final EtOH concentration was < 1 % v/v.

Stock solutions of the dyes for spectroscopic measurements were prepared in EtOH (0.10 mM) and subsequently diluted into the aqueous buffer. The stock solution of BSA was prepared by dissolving a weighed amount of the lyophilized BSA (fatty-acid free preparation) in 10 mM TRIS buffer (0.1 M NaCl, pH 7.4); the concentration of BSA diluted solutions was determined spectroscopically using the extinction coefficient value of 0.667 mg^{-1} ml cm^{-1}.[1] For the experiments described in Figure 4, BSA (96 mg) was dissolved in 3.28 ml of buffer solution (10 mM TRIS, 0.1 M NaCl, pH 7.4) and concentrated to 4.4 mM by filtration using amicon ultra-4 centrifugal fliter (30 kDa membrane cut-off; Millipore) filter.

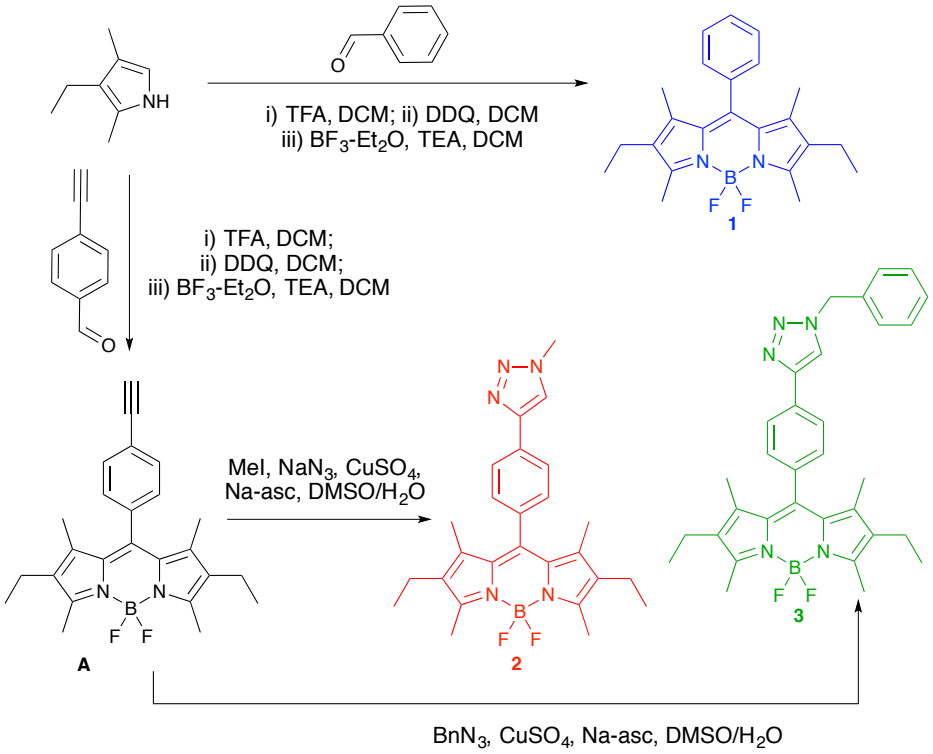
Relative quantum yields for BODIPY dyes were calculated according to the following equation:

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

where Φ_s – quantum yield of the standard; A – the absorbance (0.007 – 0.017 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 475 nm, excitation and emission slits were both set at 3 nm. Dye **1** was used as a the standard (Φ_s = 0.78 in EtOH).[5]

Synthesis and characterization of BODIPY and click-BODIPY dyes

BODIPY dyes **1, 2** and **3** were prepared according to Scheme S1.

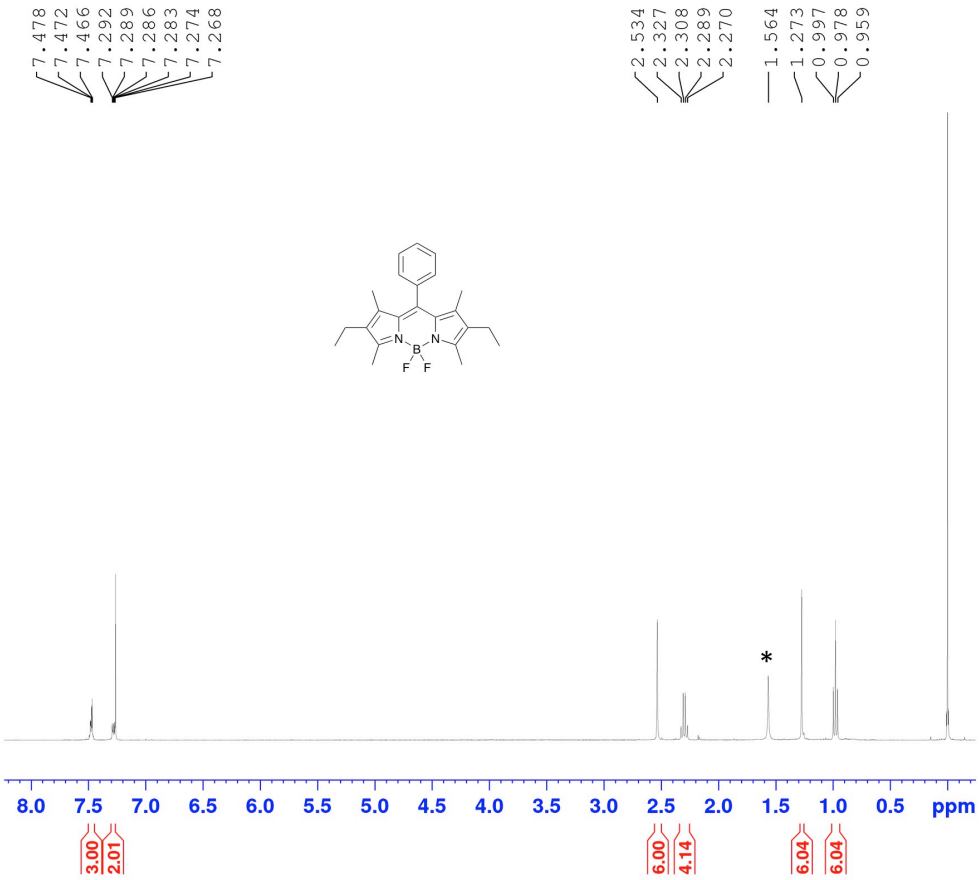


Scheme S1. Synthesis of BODIPY and click-BODIPY dyes

Dyes **1** and **3** were prepared according to literature procedures.[2,3]

Dye **2** was synthesized according to a modified literature procedure[4] as follows: a screw-cap vial that was subsequently charged with a stirring bar, DMSO (1.7 ml), alkyne-BODIPY **A** (52.4 mg, 0.129 mmol), L-proline (3.0 mg, 0.026 mmol), NaN_3 (10.1 mg, 0.156 mmol), Na_2CO_3 (2.8 mg, 0.026 mmol), sodium ascorbate (2.6 mg, 0.013mmol). Next, water (0.17 ml) and MeI (8.0 μl, 0.129 mmol) were added via syringe, followed by $CuSO_4 \cdot 5H_2O$ (1.6 mg, 0.0065 mmol). The flask was capped, parafilmed, wrapped in aluminum foil and placed in a 70°C oil bath under vigorous stirring overnight. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (20 ml) and extracted with water (2 x 10 ml), brine (10 ml), dilute NH_4OH (10 ml) and water (10 ml). The organic layer was dried over $MgSO_4$, and volatiles removed in vacuo. The residue was subjected to silica gel chromatography using EtOAc/hexane (1/1) followed by CH_2Cl_2 as the eluent to give the dye **2** (12.1 mg; 20 % yield) as a dark red solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.96 (d, *J* = 8.5 Hz, 2H), 7.84 (s, 1H), 7.35 (d, *J* = 8.5 Hz, 2H), 4.19 (s, 3H), 2.53 (s, 6H), 2.30 (q, *J* = 7.6 Hz, 4H), 1.33 (s, 6H), 0.98 (t, *J* = 7.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.07, 147.66, 139.88, 138.57, 135.86, 133.07, 131.32, 130.95, 129.15, 126.47, 121.16, 37.11, 29.93, 17.31, 14.86, 12.13; HRMS (EI): $[M]^+$ *m/z* calcd for $C_{26}H_{30}BF_2N_5$ 461.2562, found 461.2559.

^{1}H NMR spectra of dye **1**
* – H_2O in $CDCl_3$

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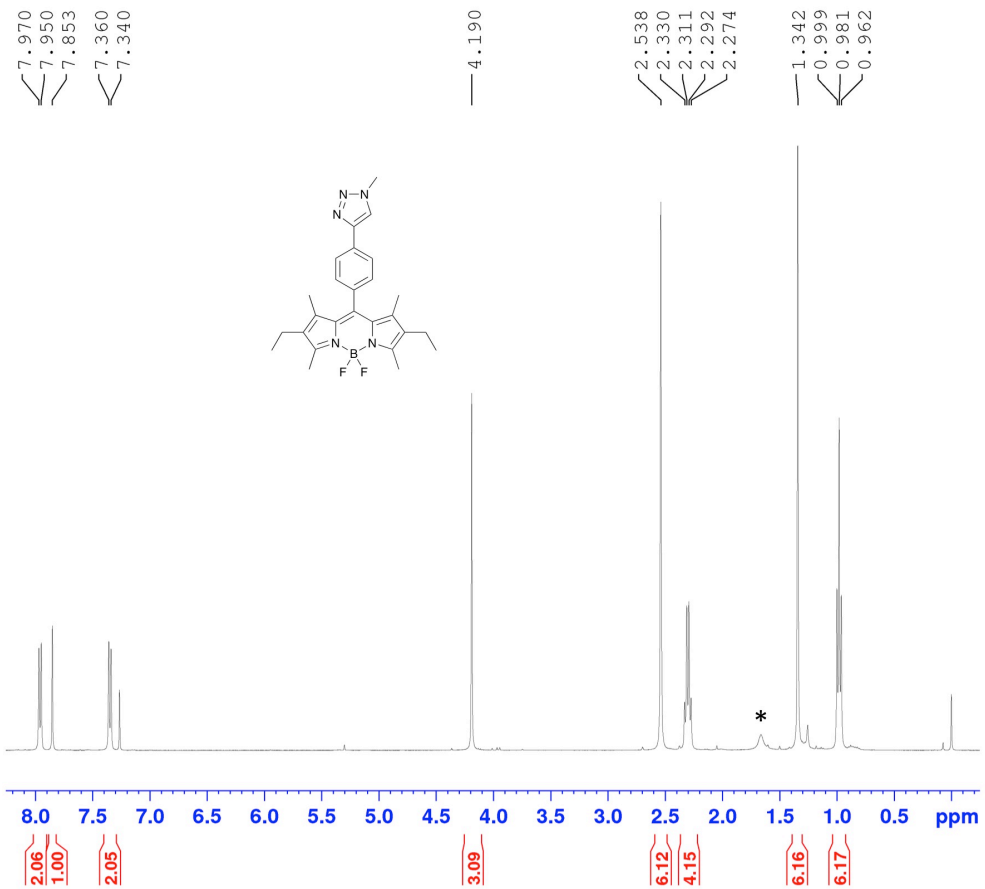
* – H_2O in $CDCl_3$

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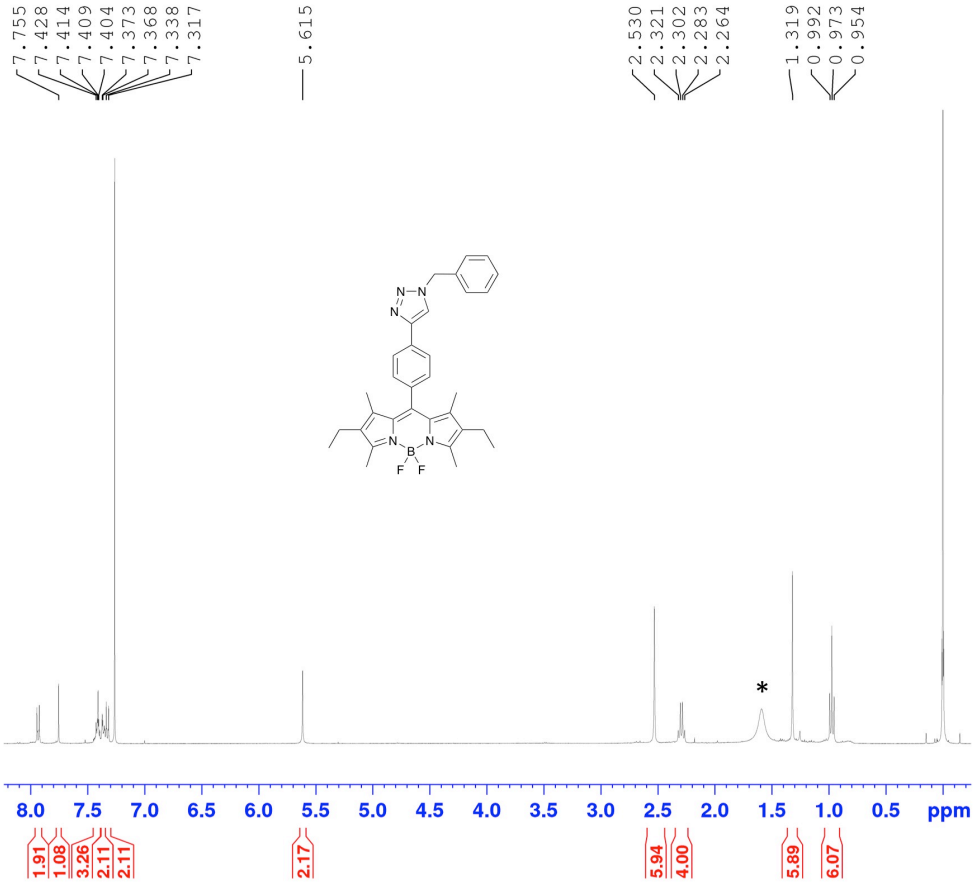
^{1}H NMR spectra of dye **3**
* – H_2O in $CDCl_3$

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NUC1                   1H
P1                  10.00 usec
PLW1         25.00300026 W

F2 - Processing parameters
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SF           400.1300084 MHz
WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00
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Table S1. Quantum yields for dyes **1**, **2** and **3**, in the presence and absence of BSA.[a]

dye	Φ
1	0.17
2	0.35
3	0.03
1 + BSA[b]	0.48
2 + BSA[b]	0.53
3 + BSA[b, c]	0.37

[a] – buffer: 10 mM TRIS buffer (0.1 M NaCl, pH 7.4), EtOH 0.1 % v/v.
[b] – [BSA] = 38 μM
[c] – dye **3** and BSA were incubated for *ca.* 30 min, prior to the measurement, since fluorescence intensity of dye **3** increased with time (the phenomenon related to the kinetics of protein-dye association and the corresponding desolvation effects of the dye and its aggregates; see Figure 3 and the accompanying paragraphs in the main text).

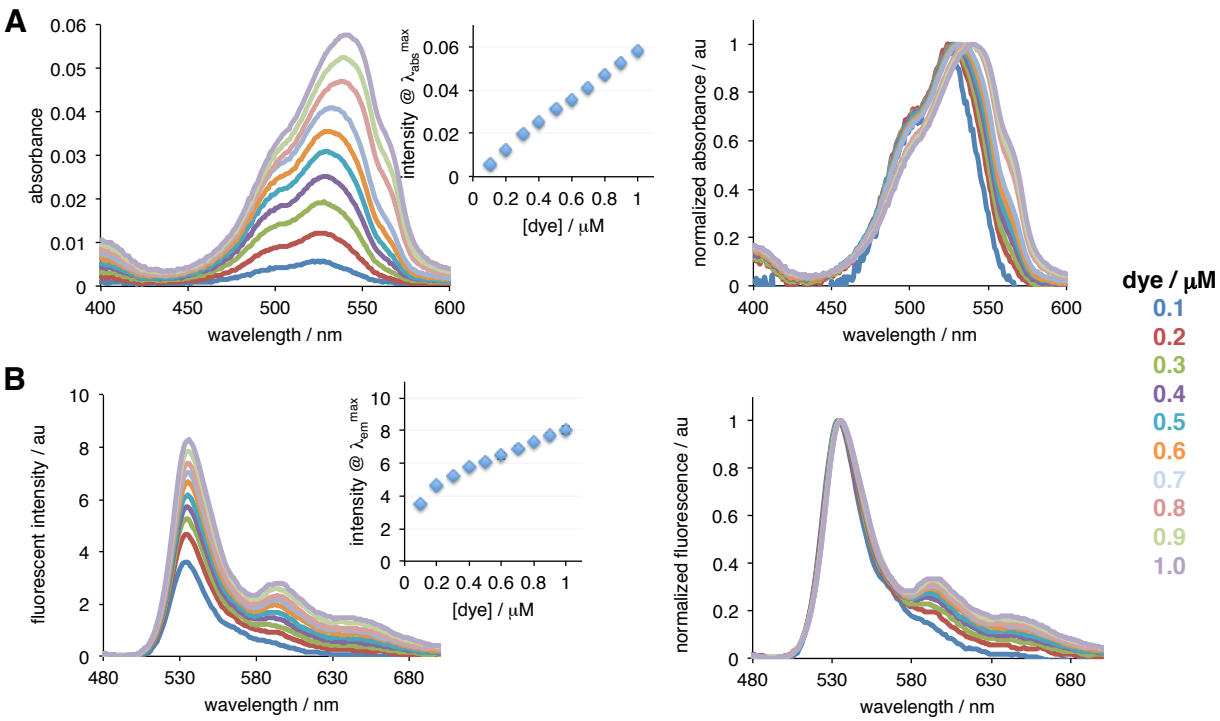

Figure S1 Spectral properties of dye **1** in buffer
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm (left) and normalized emission spectra as a function of dye's concentration (right).

[1] / μM	λ_{abs}^{max} / nm	λ_{em}^{max} / nm
0.1	522	533
0.2	525	534
0.3	528	534
0.4	529	535
0.5	529	535
0.6	529	535
0.7	529	535
0.8	538	535
0.9	539	535
1.0	540	536

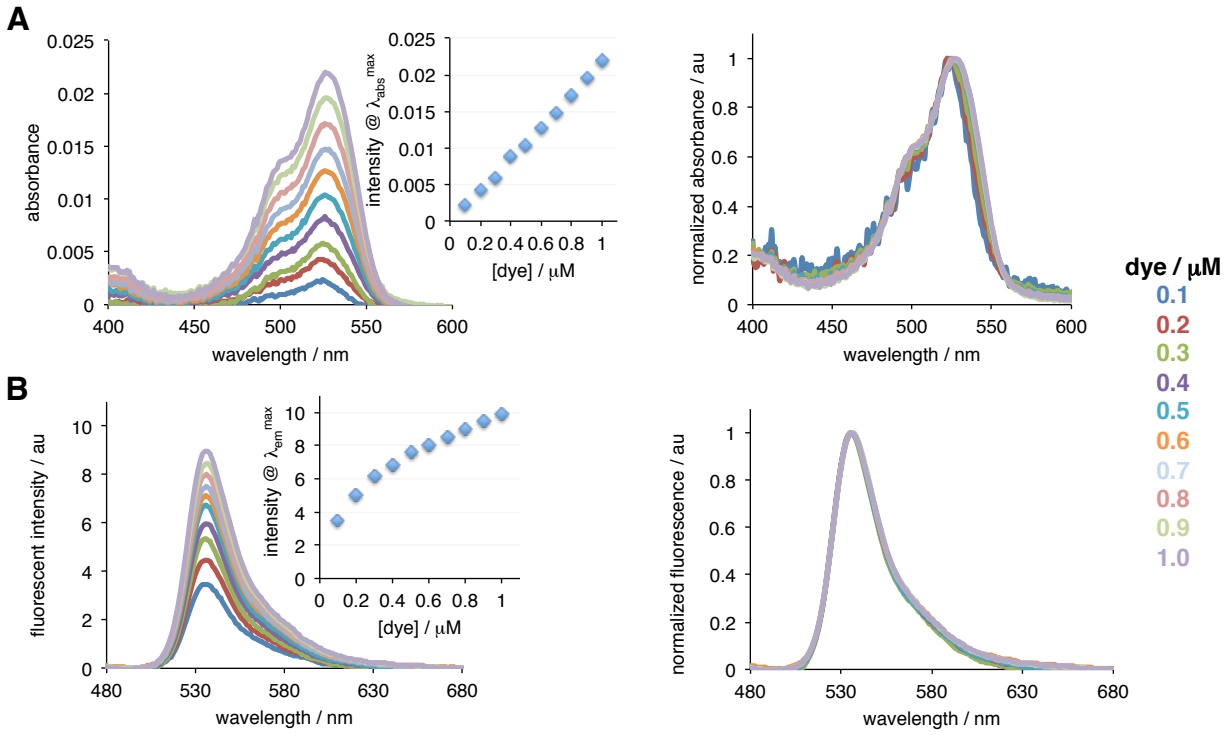

Figure S2 Spectral properties of dye **2** in buffer
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm (left) and normalized emission spectra as a function of dye's concentration (right).

[2] / μM	λ_{abs}^{max} / nm	λ_{em}^{max} / nm
0.1	524	536
0.2	522	536
0.3	525	536
0.4	526	536
0.5	526	536
0.6	526	536
0.7	526	536
0.8	526	536
0.9	526	536
1.0	526	536

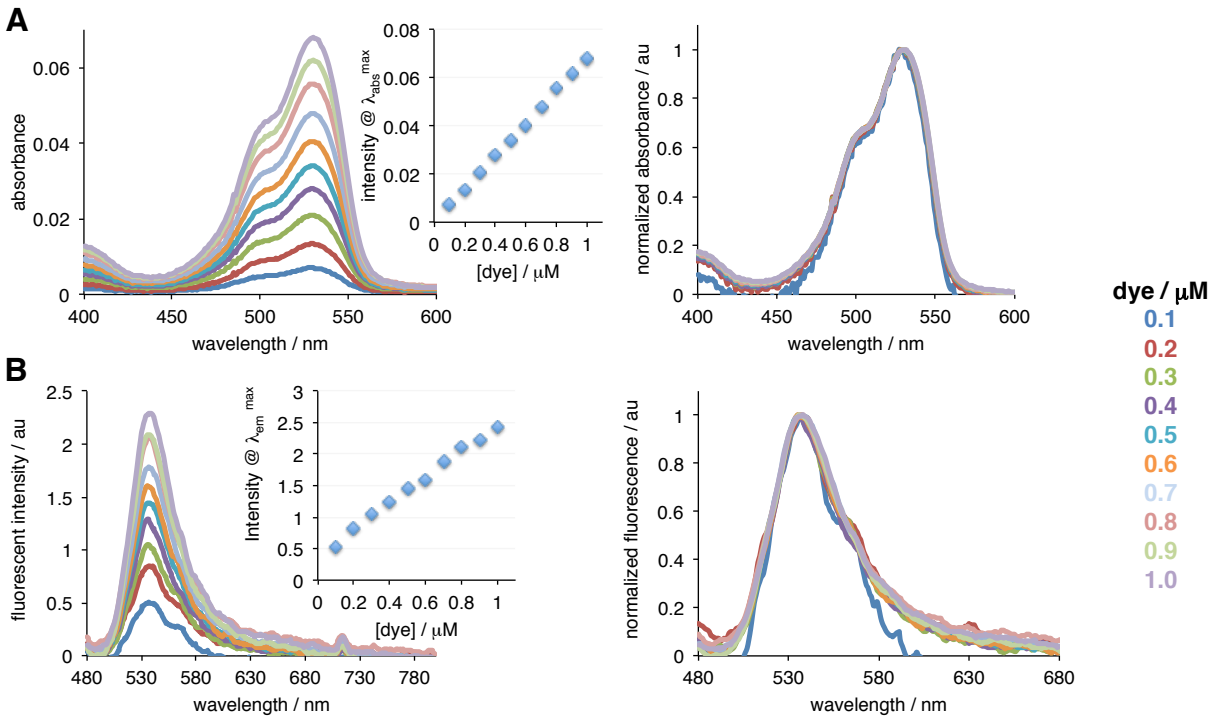

Figure S3 Spectral properties of dye **3** in buffer
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm (left) and normalized emission spectra as a function of dye's concentration (right).

[3] / μM	λ_{abs}^{max} / nm	λ_{em}^{max} / nm
0.1	527	536
0.2	529	537
0.3	529	536
0.4	529	536
0.5	529	535
0.6	529	537
0.7	529	537
0.8	530	537
0.9	530	536
1.0	530	538

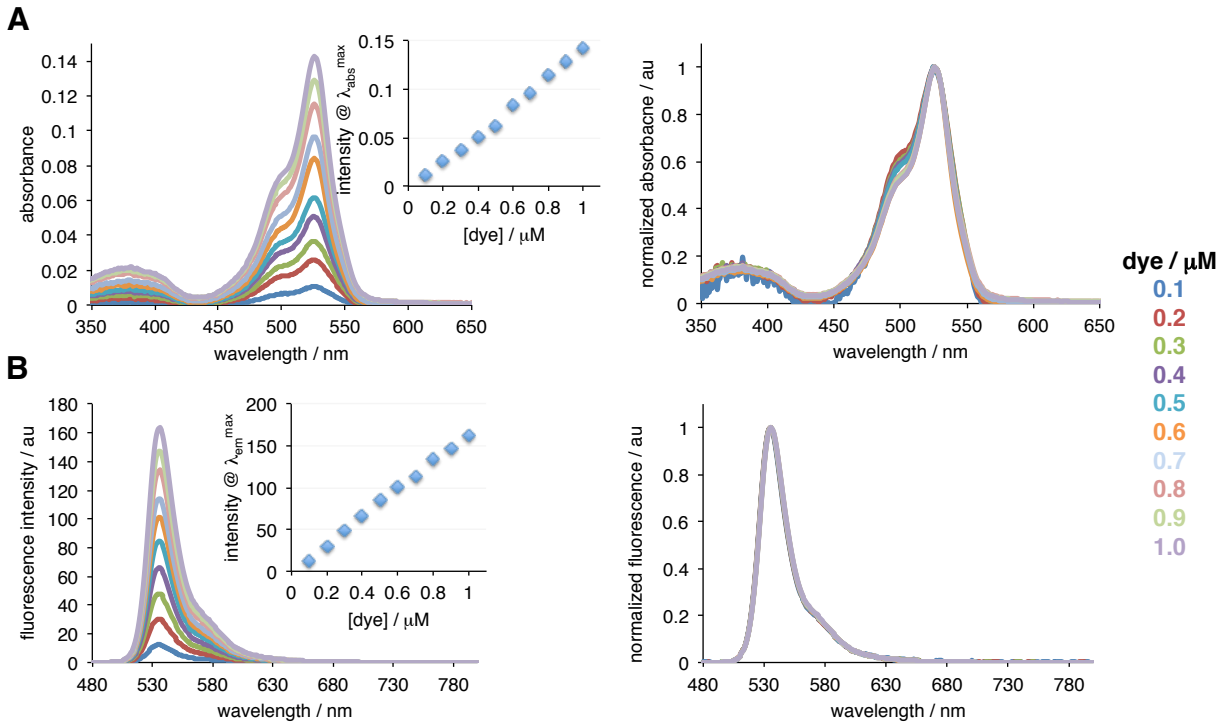

Figure S4 Spectral properties of dye **1** in the presence of BSA.
[BSA] = 39 μM
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm

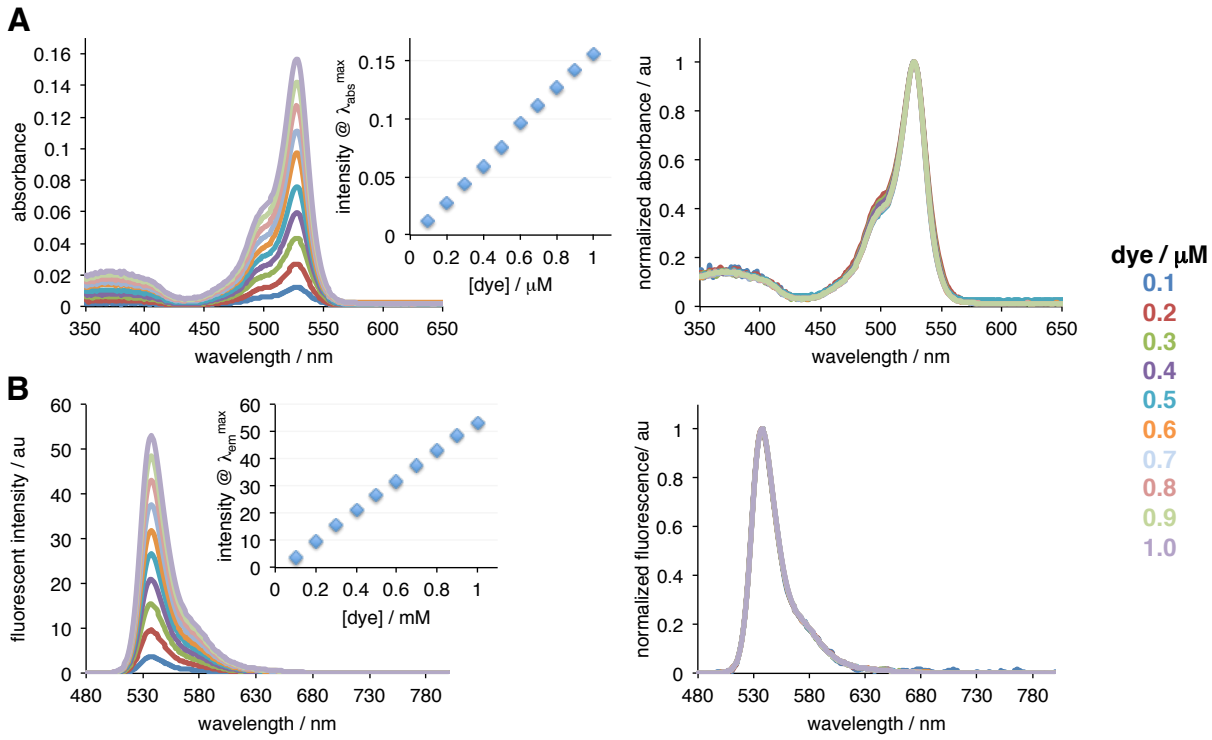

Figure S5 Spectral properties of dye **2** in the presence of BSA.
[BSA] = 39 μM
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm

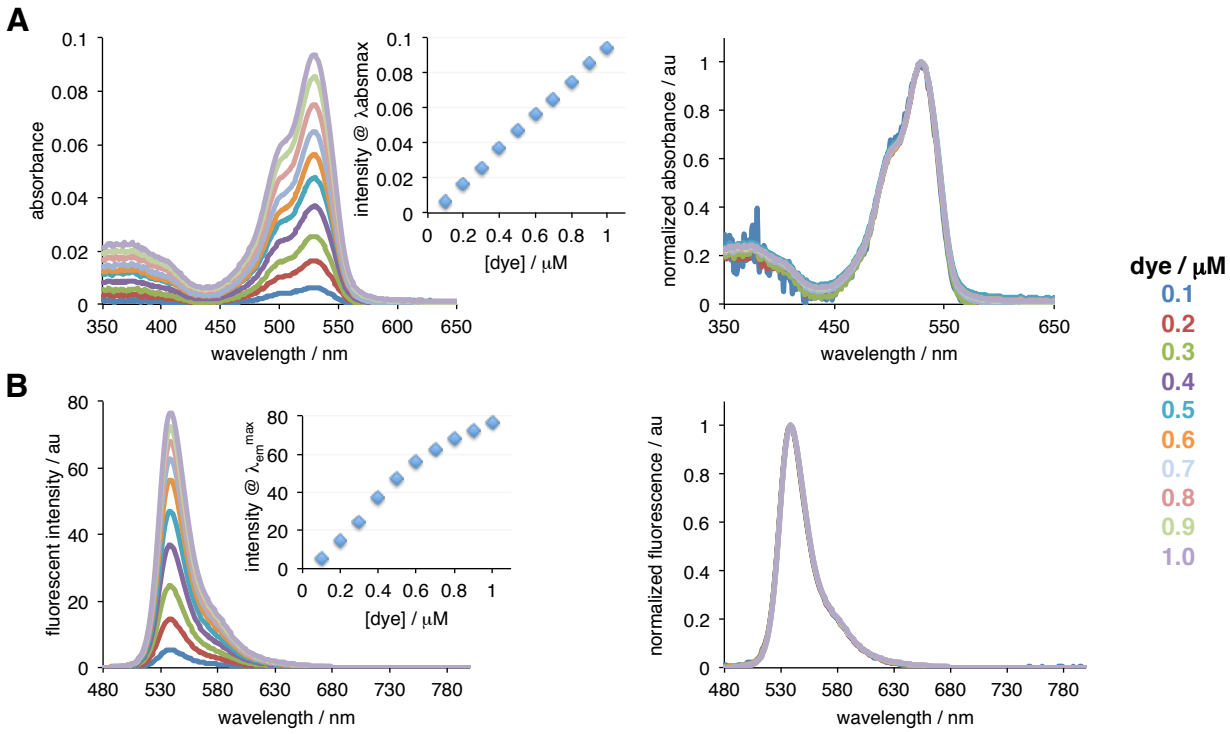

Figure S6 Spectral properties of dye **3** in the presence of BSA.
[BSA] = 39 μM
A: Absorption spectra as a function of dye's concentration (left) and normalized absorption spectra as a function of dye's concentration (right);
B: Emission spectra as a function dye's concentration; λex = 475 nm

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2. dye **1**: a) A. Haefele, G. Ulrich, P. Retailleau, R. Ziessel, *Tetrahedron Lett.*, **2008**, *49*, 3716; b) G. Ulrich, R. Ziessel, *Synlett*, **2004**, 439.

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