Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Di- and tri-oxalkyl derivatives of a boron dipyrromethene (BODIPY) rotor dye in lipid bilayers

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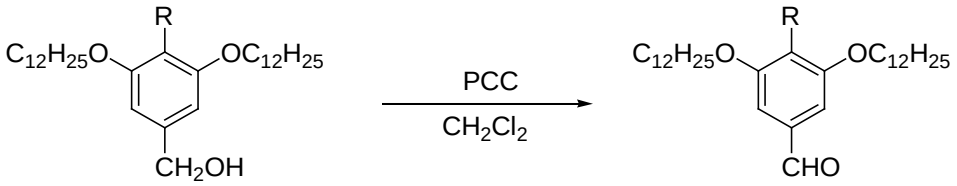
Content:

1. Synthesis of Di- and tri-oxalkyl derivatives of Boron Dipyrromethene (BODIPY) molecular rotor dyes

General information

2-Methylpyrrole was prepared according to[S1], 3,5-didodecyloxybenzaldehyde and 3,4,5-tridodecylbenzaldehyde by oxidation of the known benzyl alcohols.[S2,S3] Column chromatography was performed using 60–100 mesh silica gel (Kieselgel 60, Merck). Melting points were determined on a Boetius Leova VM TG block and are uncorrected. Structures of intermediates and final products were confirmed by ^{1}H NMR spectroscopy on Varian Gemini 300 HC instrument, 300 MHz for ^{1}H and 75 MHz for ^{13}C. Deuteriochloroform was used as solvent, the signal of the solvent served as internal standard. Chemical shifts are given in the δ-scale (ppm),

coupling constants *J* in Hz. Purity of all final compounds was confirmed by HPLC analysis (Tessek C18 25x4.5 RP column).

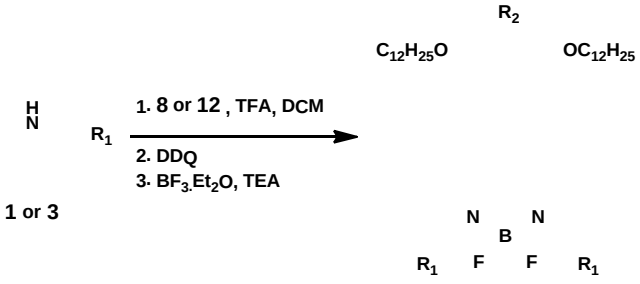


1 R=H
2 R=$OC_{12}H_{25}$

3 R=H
4 R=$OC_{12}H_{25}$

3,5-Bis(dodecyloxy)benzaldehyde (**3**)
To a solution of alcohol **1** (4.23 g; 8.92 mmol) in dry dichloromethane (150 ml), PCC (3.28 g; 15.19 mmol) was added and the reaction was stirred at RT for 1 h. Diethyl ether (100 ml) was added and the formed precipitate was filtered off. The filtrate was passed through a small column of florisil, washed with brine (10 ml) and dried with magnesium sulfate. Solvent was removed in vacuo to yield 3.96 g (83 %) of aldehyde **3** as colourless oil which solidified on standing. Spectral data of **3** are in agreement with those in ref.[S4]

3,4,5-Tris(dodecyloxy)benzaldehyde (4)
By the same method as for **3**, oxidation of alcohol **2** (9.31 g; 14.08 mmol) yielded 6.42 g (69 %) of aldehyde **4** in form of a white solid, m.p. 50 °C. m.p.[S3] 49.8 °C.



	R_1	R_2
13	H	H
14	CH_3	H
15	H	$OC_{12}H_{25}$
16	CH_3	$OC_{12}H_{25}$

10-(3,5-Bis(dodecyloxy)phenyl)-5,5-difluoro-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (**13**)
To a solution of pyrrole (7.04 g; 105 mmol) and aldehyde **3** (1.00 g; 2.10 mmol) in dry DCM (20 ml) in argon atmosphere, trifluoroacetic acid (TFA) was added (one drop) and the mixture was stirred for 2.5 h at room temperature. Subsequently DDQ (950 mg; 4.20 mmol) was added and stirring continued for 15 min. Triethylamine (2.76 ml) was added, followed by $BF_3.Et_2O$ (4 ml) and the mixture was stirred at room temperature for additional 30 min. The reaction mixture was washed with water (15 ml) and the organic phase was dried with magnesium sulfate. The

solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent toluene) to yield 174 mg (12%) of BODIPY **13** as a dark orange viscous oil. ^{1}H-NMR: 7.93 (d, *J* = 1.8 Hz, 2H, H-Ar), 7.03 (d, *J* = 4.1 Hz, 2H, H-Ar), 6.68 (d, *J* = 2.3 Hz, 2H, H-Ar), 6.64 (d, *J* = 2.2 Hz, 1H, H-Ar), 6.5 (dd, ^{3}J = 1.6 Hz, ^{3}J = 2.3 Hz, 2H, H-Ar), 3.97 (t, *J* = 6.7 Hz, 4H, 2xOCH_2), 1.8 (m, 4H, 2xCH_2), 1.36 (m, 36H, 18xCH_2), 0.9 (t, *J* = 6.7 Hz, 6H, 2xCH_3). ^{13}C-NMR ($CDCl_3$): 160.27, 147.65, 144.35, 135.52, 135.07, 131.83, 118.63, 109.46, 103.76, 68.65, 32.15, 29.90, 29.86, 29.83, 29.80, 29.62, 29.58, 29.42, 26.27, 22.92, 14.35. MS $[M+Na]^+$ = 659.454.

10-(3,5-Bis(dodecyloxy)phenyl)-5,5-difluoro-3,7-dimethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (**14**)

To a solution of 2-methylpyrrole (2.55 g; 21.59 mmol) and aldehyde **3** (2.00 g; 4.21 mmol) in dry DCM (100 ml) in argon atmosphere, trifluoroacetic acid (TFA) was added (one drop) and the mixture was stirred for 3 h at room temperature. Subsequently DDQ (955 mg; 4.21 mmol) was added and stirring continued for 15 min. Triethylamine (5.75 ml) was added, followed by $BF_3.Et_2O$ (8.13 ml) and the mixture was stirred at room temperature for additional 30 min. The reaction mixture was washed with water (30 ml) and the organic phase was dried with magnesium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent toluene) to yield 969 mg (27%) of BODIPY **14**, m.p. 58-59 °C. ^{1}H-NMR: 6.82 (d, *J* = 4.1 Hz, 2H, H-Ar), 6.62 (d, *J* = 2.1 Hz, 2H, H-Ar), 6.60 (dd, ^{4}J = 2.1 Hz, ^{4}J = 2.1 Hz, 1H, H-Ar), 6.26 (d, *J* = 4.1 Hz, H-Ar), 3.97 (t, *J* = 6.7 Hz, 4H, 2xOCH_2), 2.65 (s, 6H, 2xCH_3), 1.8 (m, 4H, 2xCH_2), 1.31 (m, 36H, 18xCH_2), 0.89 (t, *J* = 6.7 Hz, 6H, 2xCH_3). ^{13}C-NMR ($CDCl_3$): 160.08, 157.79, 142.81, 135.89, 134.60, 130.62, 119.46, 109.36, 103.07, 68.55, 32.17, 29.92, 29.89, 29.85, 29.82, 29.64, 29.60, 29.45, 26.28, 22.94, 15.12, 14.36. MS $[M+H]^+$ = 665.502.

5,5-Difluoro-10-(3,4,5-tris(dodecyloxy)phenyl)-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (**15**)

To a solution of pyrrole (2.55 g; 38.06 mmol) and aldehyde **4** (500 mg; 0.76 mmol) in dry DCM (10 ml) in argon, trifluoroacetic acid (TFA) was added (one drop) and the mixture was stirred for 1 h at room temperature. Subsequently a solution of DDQ (172 mg; 0.76 mmol) in dry DCM (10 ml) was added and stirring continued for 10 min. Triethylamine (1 ml) was added, followed by $BF_3.Et_2O$ (1.47 ml) and the mixture was stirred at room temperature for additional 30 min. The reaction mixture was washed with water (5 ml) and the organic phase was dried with magnesium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent toluene). 55 mg (9%) of BODIPY **15** was isolated, m.p: 42-44 °C ^{1}H-NMR: 7.91 (d, *J* = 1.8 Hz, 2H, H-Ar), 7.02 (d, *J* = 4.1 Hz, 2H, H-Ar), 6.78 (s, 2H, H-Ar), 6.53 (dd, ^{3}J = 4.1 Hz, ^{3}J = 1.8 Hz, 2H, H-Ar), 4.08 (t, *J* = 6 Hz, 2H, OCH_2), 3.99 (t, *J* = 6 Hz, 4H, 2xOCH_2), 1.81 (m, 6H, 3xOCH_2**CH_2**), 1.60-1.20 (m, 54H, 27xCH_2), 0.88 (m, 9H, 3xCH_3). ^{13}C-NMR ($CDCl_3$): 153.18, 143.94, 140.95, 135.05, 131.71, 128.88, 118.57, 109.76, 73.94, 69.64, 32.17,

32.15, 30.63, 30.00, 29.92, 29.88, 29.86, 29.64, 29.59, 26.32, 22.92, 14.34. MS $[M+Na]^+$ = 843.636.

5,5-Difluoro-3,7-dimethyl-10-(3,4,5-tris(dodecyloxy)phenyl)-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (**16**)

To a solution of 2-methyl-1*H*-pyrrole (887 mg; 10.9 mmol) and aldehyde **4** (3.61 g; 5.48 mmol) in dry DCM (100 ml) in argon atmosphere, trifluoroacetic acid (TFA) was added (one drop) and the mixture was stirred for 1.5 h at room temperature. Subsequently a solution of DDQ (1.24 g; 5.48 mmol) in dry DCM (30 ml) was added and stirring continued for 15 min. Triethylamine (7.48 ml) was added, followed by $BF_3.Et_2O$ (10.56 ml) and the mixture was stirred at room temperature for additional 30 min. The reaction mixture was washed with water (20 ml), brine (10 ml) and the organic phase was dried with magnesium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (eluent toluene). Yield 1.80 g (38%) of BODIPY **16**, m.p. 57-59 °C ^{1}H-NMR: 6.86 (d, *J* = 4.1 Hz, 2H, H-Ar), 6.30 (d, *J* = 4.1 Hz, 2H, H-Ar), 4.11 (t, *J* = 6 Hz, 2H, OCH_2), 4.02 (t, *J* = 6 Hz, 4H, 2xOCH_2), 2.69 (s, 6H, 2xCH_3-Ar), 1.85 (m, 6H, 3xOCH_2**CH_2**), 1.61-1.25 (m, 54H, 27xCH_2), 0.91 (m, 9H, 3xCH_3). ^{13}C-NMR: 157.54, 153.03, 143.14, 140.13, 134.68, 130.68, 130.56, 129.27, 119.47, 109.58, 77.76, 77.35, 76.92, 73.86, 69.55, 32.25, 32.23, 30.71, 30.08, 30.06, 30.01, 29.97, 29.94, 29.92, 29.73, 26.43, 26.40, 23.00, 15.12, 14.40. MS $[M+H]^+$ = 849.685.

References

S1. P. A. Liddel, T. P. Forsyth, M. O. Senge, K. M. Smith: *Tetrahedron* **49**, 1343-1350 (1993).
S2. B. Helms, C. O. Liang, C. J. Hawker, J. M. Frechet: *Macromolecules* **38**, 5411-5415 (2005).
S3. W.-R. Li, Y. Kwo-Cheng, L. Ying-Chih, K. Chung: *Helv. Chim. Acta* **82**, 1400-1407 (1999).
S4. G. Zerban, H. Meier: *Z. Naturforsch. B*, **48**, 171-184 (1993).

2. Localisation of the reference probe DiD in the lipid bilayer.

DiD is a fluorescence probe with two acyl chains that are connected to the DiD chromophore. Intuitively, one would expect DiD chromophore to reside at the lipid-water interface. To confirm this, we have performed FRET experiments similar to those that are shown in the main text and analysed fluorescence decays (Figure S4) by means of the Baumann-Fayer (BF) model. Dihexanoylphosphatidylethanolamine (DHPE) labeled in the headgroup region with BODIPY (BODIPY FL-DHPE; Life Technologies) was used as the donor while DiD was used as the acceptor. Fitting the data by BF model yielded an excellent fit and the following output parameters: plane to plane distance, *i.e.* transversal distance from the plane of donors to the plane of acceptors corresponding roughly to the bilayer thickness (*cf.* Fig. 3), d_B = 40.6 Å and the acceptor surface concentration C_A = 1.14. The latter parameter agrees well with the expected surface concentration C_A = 1.26, which is determined by probe and lipid mixing ratio. Since BODIPY that

is attached to DHPE in the headgroup region must reside at the lipid-water interface and d_B corresponds well to the bilayer thickness, it can be concluded that DiD is localised at the lipid-water interface as well.

3. Supplementary Figures S1- S4

Legends

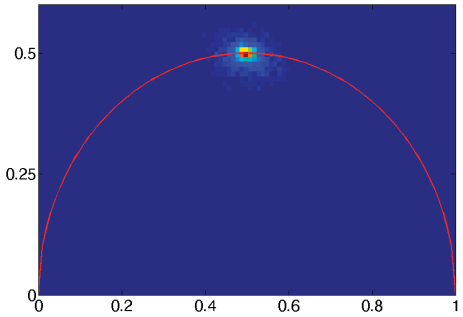
Figure S1. a) Illustrative phasor plots depicting mono-exponential (upper panel) and multi-exponential (lower panel) fluorescence lifetime decays. b) Phasor plots depicting experimental fluorescence lifetime data measured for compounds 1 and 2b (upper panel) and 1 and 3b (lower panel) in GUVs composed of DPPC (So phase) measured at room temperature.

Figure S2. a) Fluorescence intensity image and b) fluorescence lifetime map of GUVs composed of DOPC (Ld phase) and DPPC (So phase) measured at room temperature. Fluorescence lifetime map was generated by pseudo-colouring based on the selection using phasor plot analysis (c, blue and red circle).

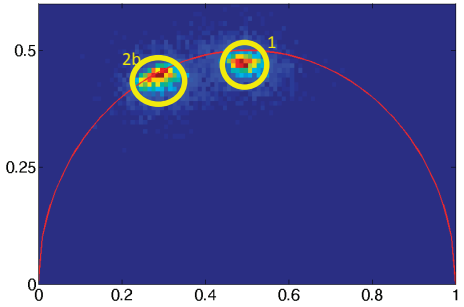
Figure S3. Pseudo-colour fluorescence intensity image of GUV composed of DOPC (Ld phase) measured at room temperature. Colour-bar indicates intensity values in the image.

Figure S4. Time-resolved fluorescence decays of DHPE-BODIPY donors in the absence (dashed) and presence (solid) of DiD acceptors, the best fit of the latter curve (solid red) and the lamp profile (dotted) are also included.

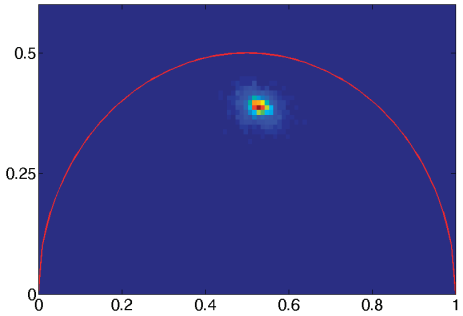
a Monoexponential decay



b Compounds 1 vs 2b



Multiexponential decay



Compounds 1 vs 3b

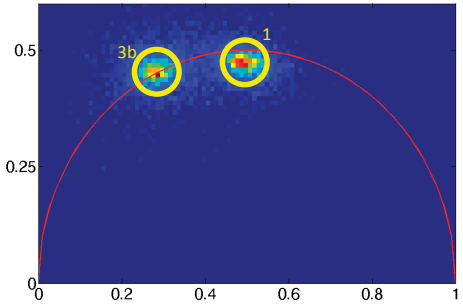


Figure S1

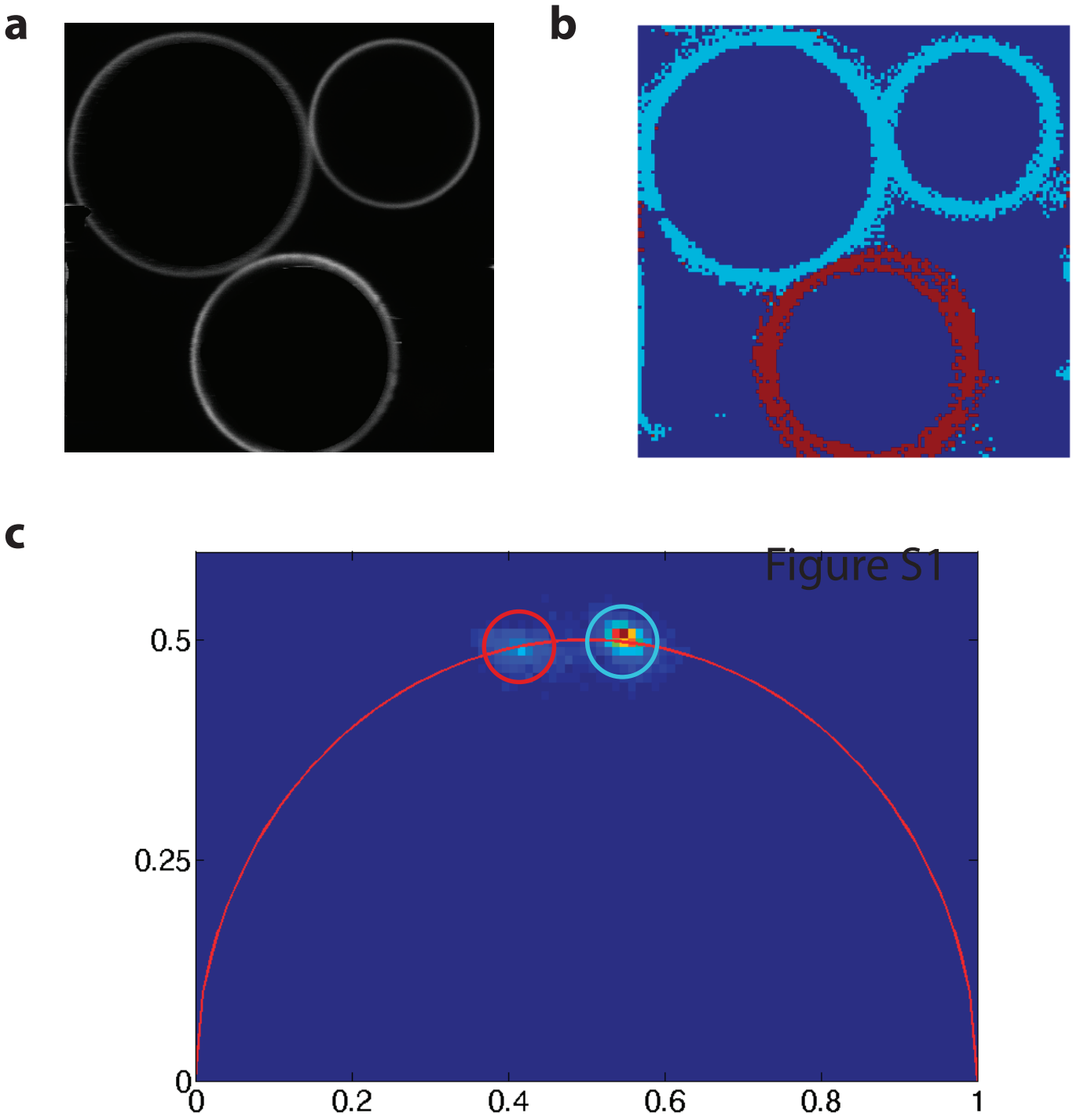


Figure S2

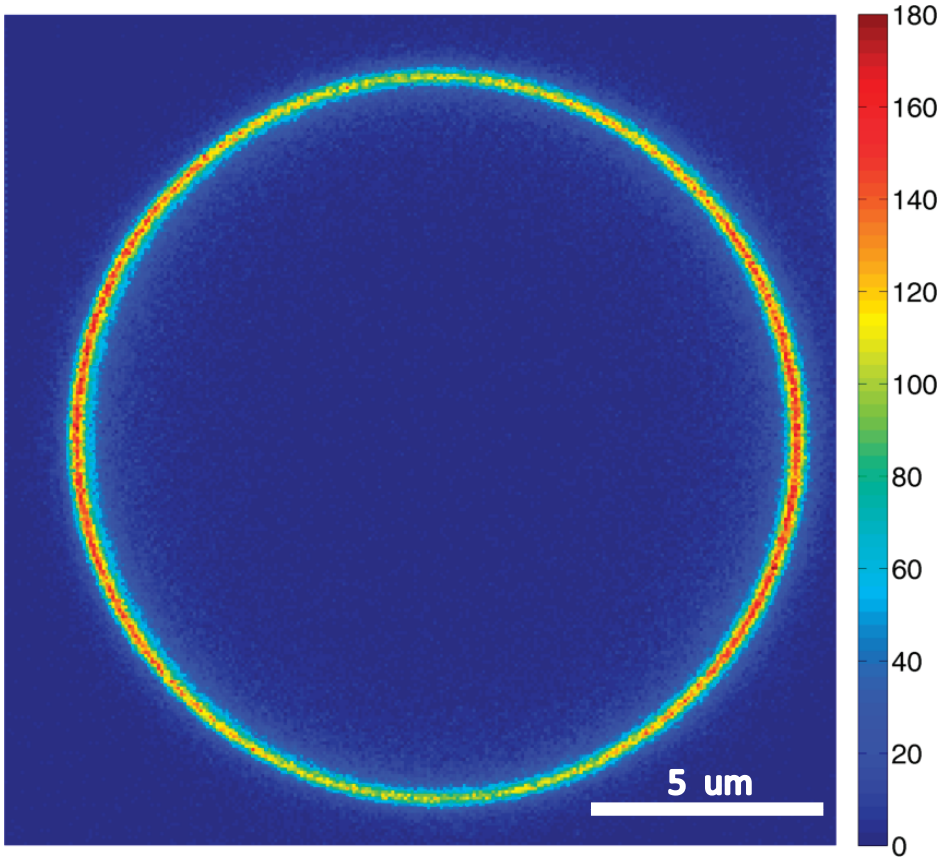


Figure S3

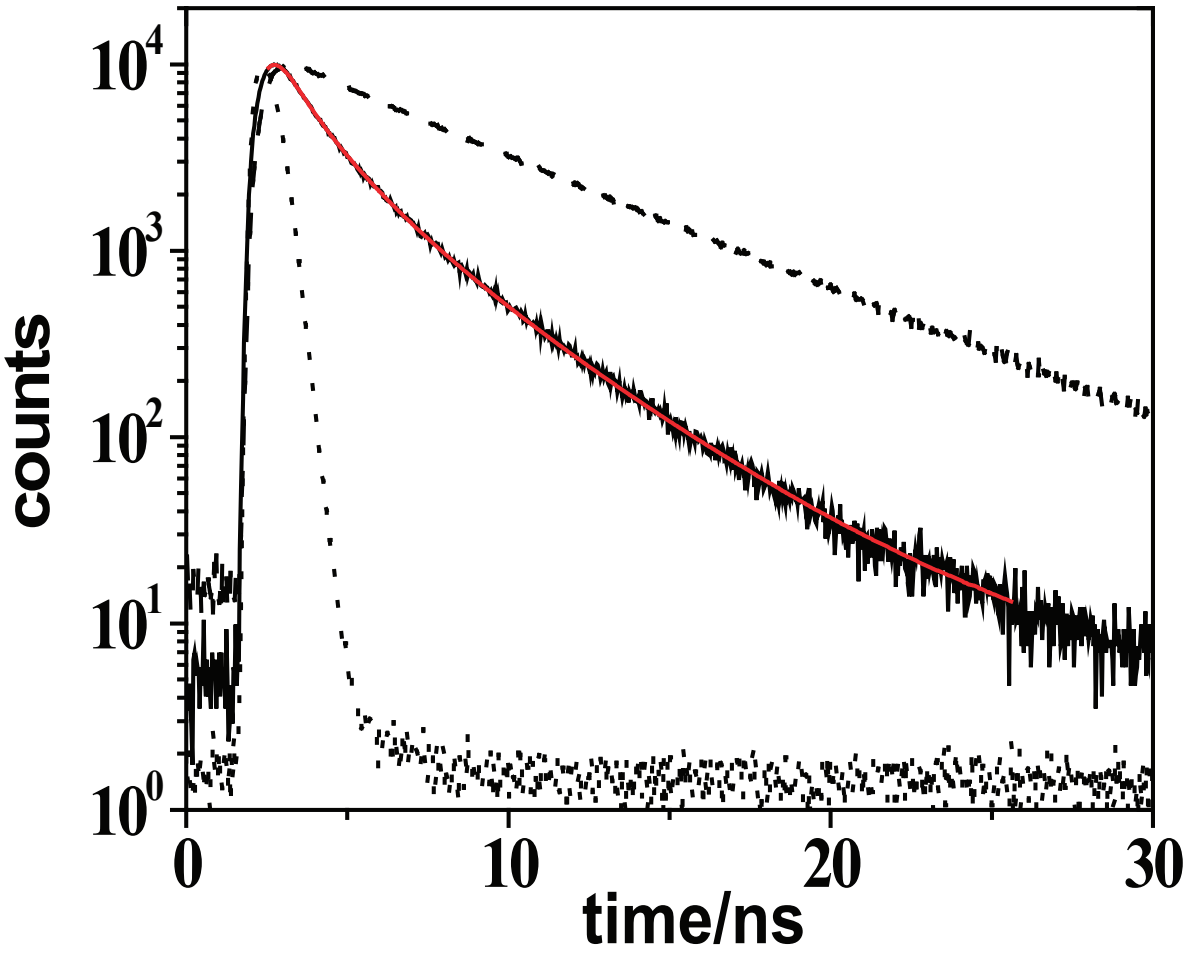


Figure S4