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SUPPLEMENTARY INFORMATION

Polar ammoniostyryls easily converting a clickable lipophilic BODIPY in an advanced plasma membrane probe

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Table of contents

1. Synthetic procedures **S2**

2. ^{1}H and ^{13}C NMR spectra **S3**

3. Photophysical properties **S4**

4. Mitochondrial imaging **S5**

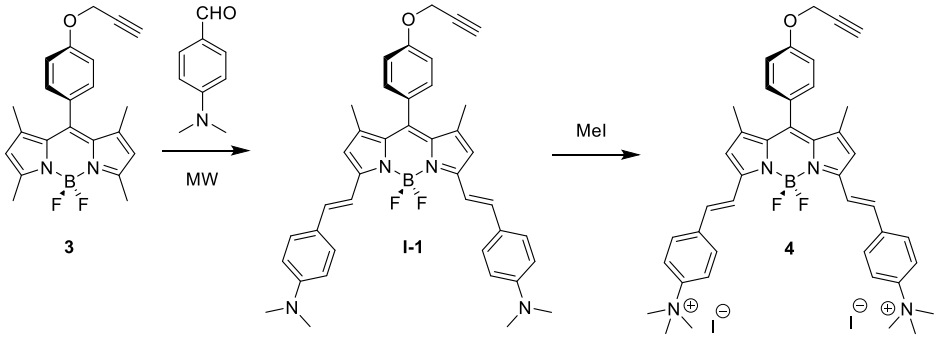
5. References **S5**

1. Synthetic procedures and characterization data

Synthesis of BODIPY 3

1,3,5,7-Tetramethyl-8-[(4-propargyloxy)phenyl]-*F*-BODIPY (**3**) was obtained in two steps from 2,4-dimethylpyrrole, *p*-hydroxybenzaldehyde and propargyl bromide (44 % overall yield) according to the procedure previously described by Zhou and col.[1] Characterization data are coincident with the described ones.[1]

Synthesis of BODIPY 4



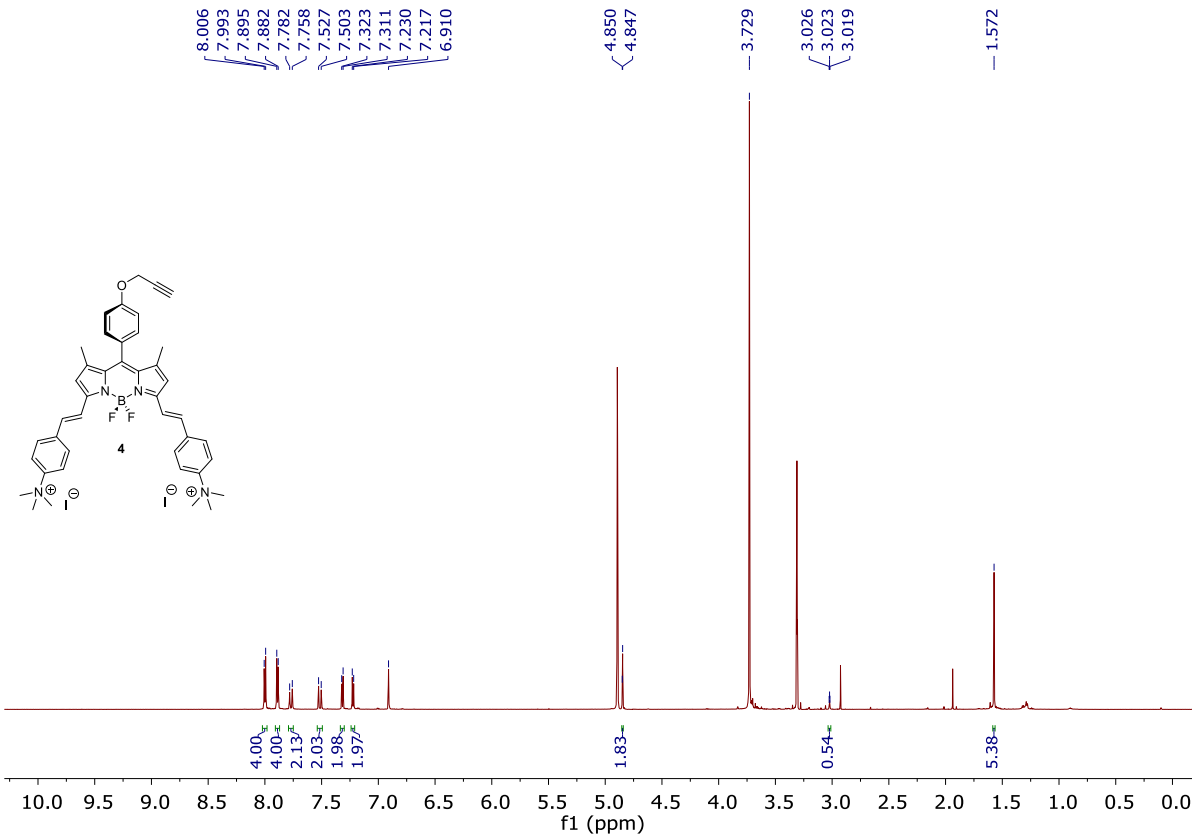
Scheme S1. Synthetic route.

Intermediate I-1. A mixture of **3** (60.0 mg, 0.159 mmol), 4-(dimethylamino)benzaldehyde (71.4 mg, 0.479 mmol), acetic acid (46.5 mg, 0.774 mmol) and piperidine (65.8 mg, 0.773 mmol) in dry DMF (1 mL) was submitted to microwave (MW) irradiation for 60 min at 120 °C. Then, the mixture was diluted with CH_2Cl_2 (20 mL), washed with water (5×20 mL) and dried over anhydrous Na_2SO_4. After filtration and solvent evaporation under reduced pressure, the obtained residue was submitted to flash chromatography (silica gel; hexane/ethyl acetate 8:2) to obtain **I-1** (75.4 mg, 74%) as a blue solid. Characterization data are coincident with those previously described by Yesilot and col.[2]

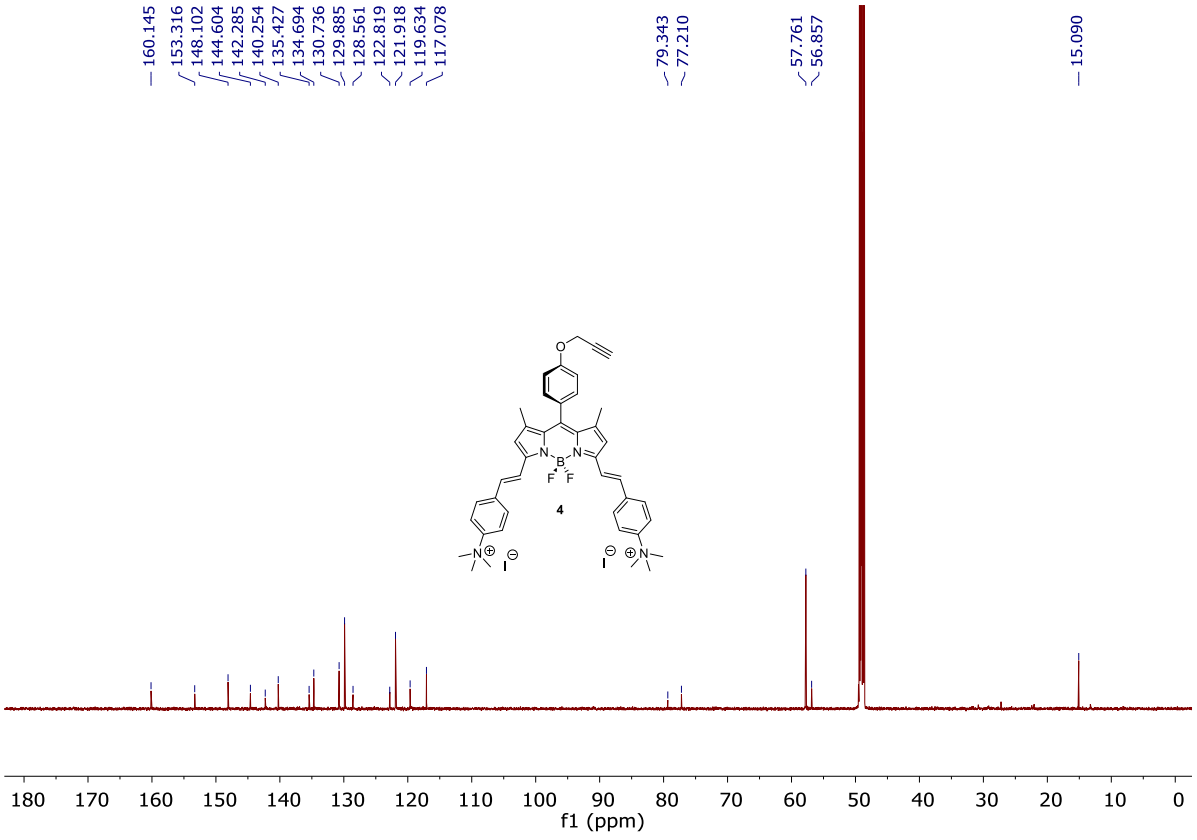
BODIPY 4. Iodomethane (1 mL) was added to a solution of **I-1** (60.0 mg, 0.094 mmol) in acetonitrile (1 mL). The resulting mixture was stirred under argon for 72 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (neutral alumina; acetonitrile/water 9:1) to obtain **4** (56.3 mg, 65%) as a blue solid. Mp: >350 °C. ^{1}H NMR (MeOH-d_4, 700 MHz) δ 8.00 (d, *J* = 8.9 Hz, 4H), 7.89 (d, *J* = 8.9 Hz, 4H), 7.77 (d, *J* = 16.4 Hz, 2H), 7.52 (d, *J* = 16.3 Hz, 2H), 7.32 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 6.91 (s, 2H), 4.85 (d, *J* = 2.2 Hz, 2H), 3.73 (s, 18H), 3.02 (t, *J* = 2.4 Hz, 1H), 1.57 (s, 6H) ppm. ^{13}C NMR (MeOH-d_4, 176 MHz) δ 160.1 (C), 153.3 (C), 148.1 (C), 144.6 (C), 142.3 (C), 140.3 (C),135.4 (C), 134.7 (CH), 130.7 (CH), 129.9 (CH), 128.6 (C), 122.8 (CH), 121.9 (CH), 119.6 (CH), 117.1 (CH), 79.3 (C), 77.2 (CH), 57.8 (CH_3), 56.9 (CH_2), 15.1 (CH_3) ppm. FTIR μ 2980, 1659, 1481, 1159, 987 cm^{-1}. HRMS (ESI$^+$/Q-TOF) *m/z*: $[M]^{+2}$ calcd. for $C_{42}H_{45}BF_2N_4O$ 335.1822; found 335.1821. HRMS (ESI$^-$/Q-TOF) *m/z*: $[M]^-$ calcd. for iodide ion 126.9039; found 126.9028.

2. ^{1}H and ^{13}C NMR spectra of BODIPY 4

^{1}H NMR (MeOH-d_4, 700 MHz) of 4



^{13}C NMR (MeOH-d_4, 176 MHz) of 4



3. Photophysical properties

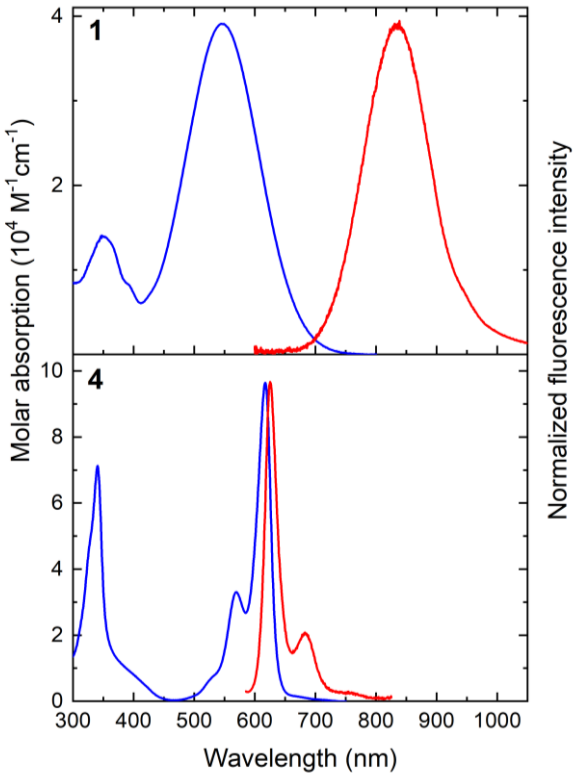


Figure S1. Absorption (blue) and normalized fluorescence (red) spectra of commercial PM probe **1** (FM 4-64, λ_{exc} = 550 nm) and BODIPY-based PM probe **4** (λ_{exc} = 580 nm) in methanol (2 μM).

Table S1. Photophysical properties of commercial PM probe **1** (FM 4-64) and BODIPY-based PM probes **2** and **4** (2 μM) in alcohols and water, as an approach to the aqueous physiological media.

Dye	Solvent	λ_{ab} [a] (nm)	ε [b] (10^4 M^{-1} cm^{-1})	λ_{em} [c] (nm)	ϕ [d]	B [e] (10^4 M^{-1} cm^{-1})	τ [f] (ns)
1	methanol	547.0	3.9	838.5	0.066	0.250	0.20
	water	490.0	1.1	830.0	0.007	0.008	-
2[g]	ethanol	582.0	11.1	606.0	0.810	8.900	-
	water	594.0	4.4	615.0	0.010	0.040	-
4	methanol	617.0	9.6	626.0	0.680	6.500	4.17
	water	617.0	3.2	626.5	0.370	1.200	3.22

[a]Maximum absorption wavelength; [b]Maximum molar absorption; [c]Maximum fluorescence wavelength; [d]Fluorescence quantum yield; [e]Fluorescence brightness ($B = \varepsilon \phi$); [f]Fluorescence lifetime; [g]Data collected from ref. 3 (lifetimes were not reported).

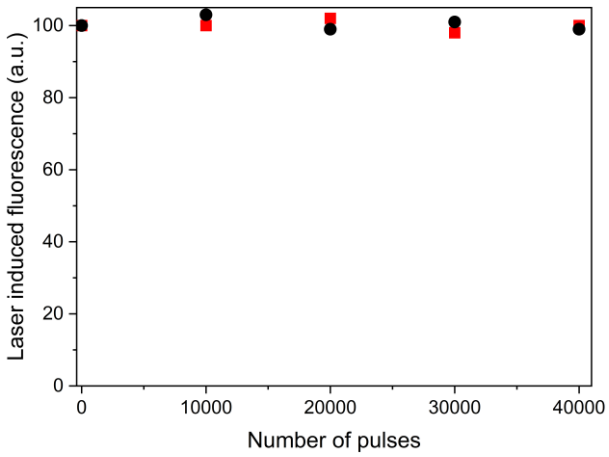


Figure S2. LIF intensity evolution of commercial PM probe **1** (FM 4-64; red) and BODIPY-based PM probe **4** (black) in methanol upon laser pumping.

4. Mitochondrial imaging

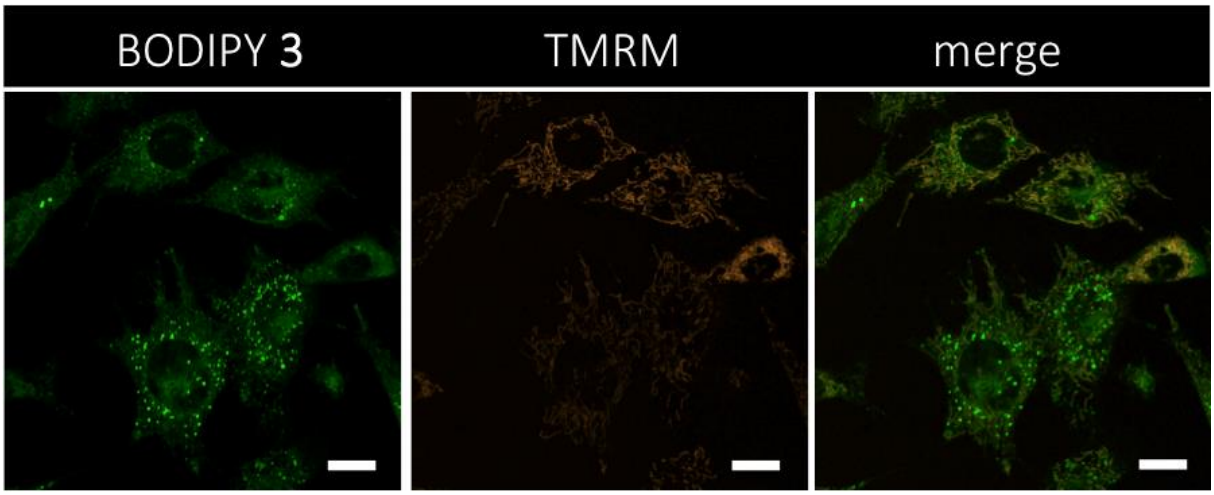


Figure S3. Dual-color imaging of MEFs with BODIPY **3** (left), TMRM (middle) and merged imaging (right). The mitochondrial network was labeled with both probes. Probes were incubated for 8 h. Final concentration of BODIPY **3** and TMRM was 200 nM and 1 µM, respectively. Green channel: λ_{exc} = 488 nm, λ_{em} = 525 ± 25 nm. Red channel: λ_{exc} = 561 nm, λ_{em} > 561 nm. R = 0.62 (Pearson´s coefficient; estimated from the whole image). See main text for details. Scale bars are 10 µm.

5. References

[1] J. Park, D. Feng, H.-C. Zhou, *J. Am. Chem. Soc.* **2015**, *137*, 1663-1672.

[2] S. Çetindere, S. O. Tümay, A. Kiliç, M. Durmus, S. Yesilot, *Dyes Pigments* **2017**, *139*, 517-523.

[3] X. Zhang, C. Wang, L. Jin, Z. Han, Y. Xiao, *ACS Appl. Mater. Interfaces* **2014**, *6*, 12372-12379.