

Supporting information

Redesigning solvatochromic probe Laurdan for imaging lipid order selectively in cell plasma membranes

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EXPERIMENTAL

Synthesis of quencher dodecyl viologen (Viologen-C12). (Adapted from¹)

1-Dodecyl-4-(1-dodecylpyridin-1-ium-4-yl)pyridin-1-ium iodide. 0.63 g of 4,4'-bipyridine were dissolved in 20 mL of dry acetonitrile together with 3.59 g (3 mL, 3 equiv.) of n-dodecyl iodide. The reaction mixture was refluxed for 20h (control by TLC). After the reaction the mixture was diluted twice with diethyl ether and the solids were filtered off and washed with Et₂O on a glass filter. The crude product was recrystallized from EtOH:H₂O 1:1 mixture. Yield 2.45 g (81%) as lustrous reddish crystals. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.38 (d, *J*=6.5 Hz, 4 H) 8.78 (d, *J*=6.5 Hz, 4 H) 4.68 (t, *J*=7.4 Hz, 4 H) 1.97 (br t, *J*=6.3 Hz, 4 H) 1.34 - 1.19 (m, 36 H) 0.85 (t, *J*=6.8 Hz, 6 H).

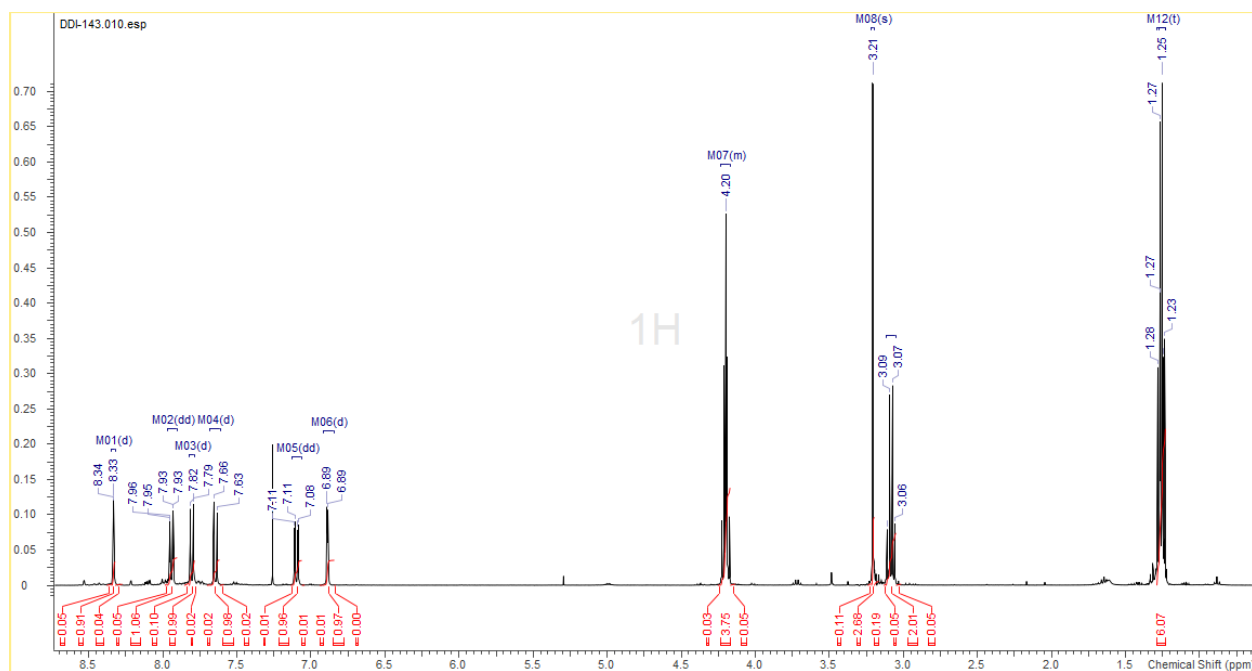


Figure S1. ¹H NMR spectrum of 2a in CDCl₃.

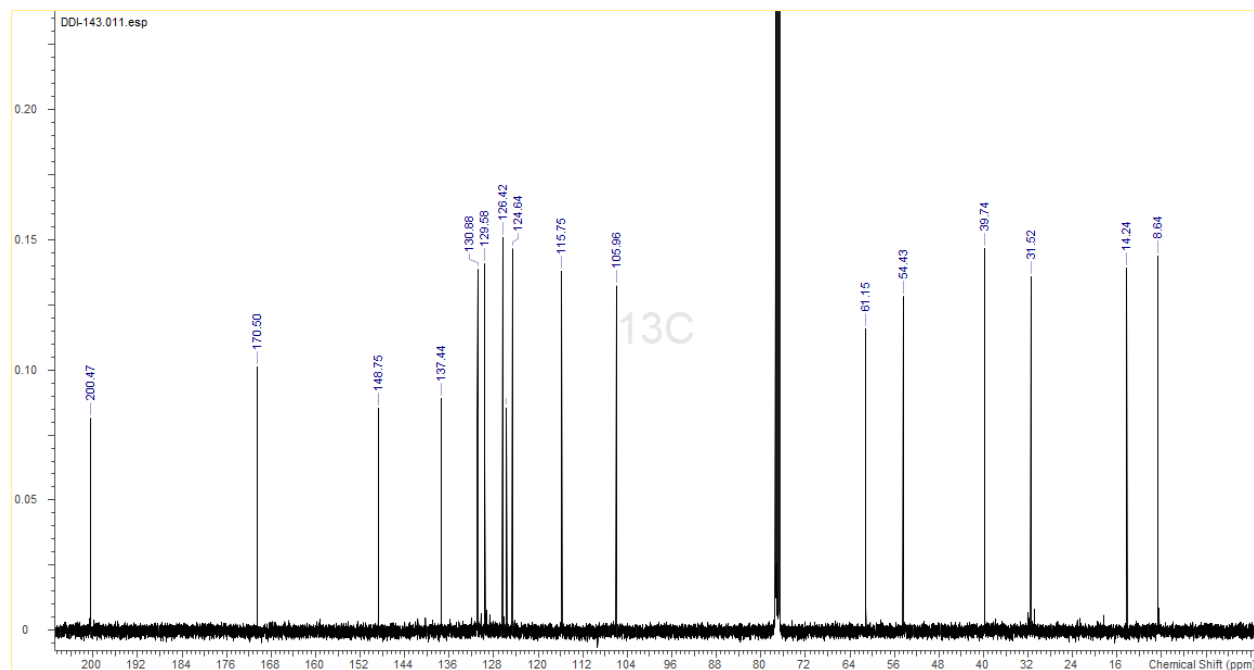


Figure S2. ^{13}C NMR spectrum of **2a** in CDCl_3 .

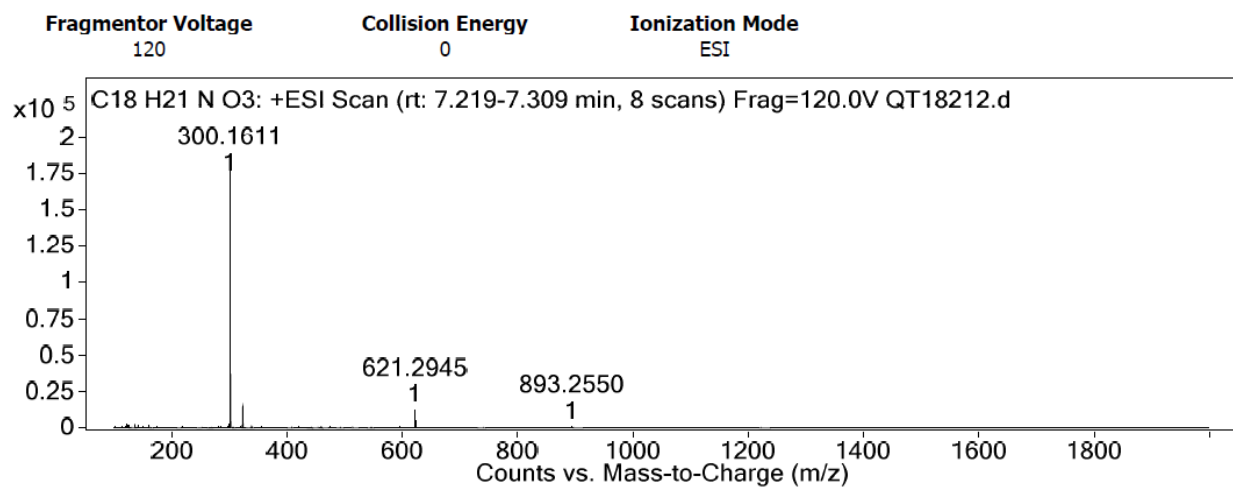


Figure S3. HRMS (ESI+) of **2a**.

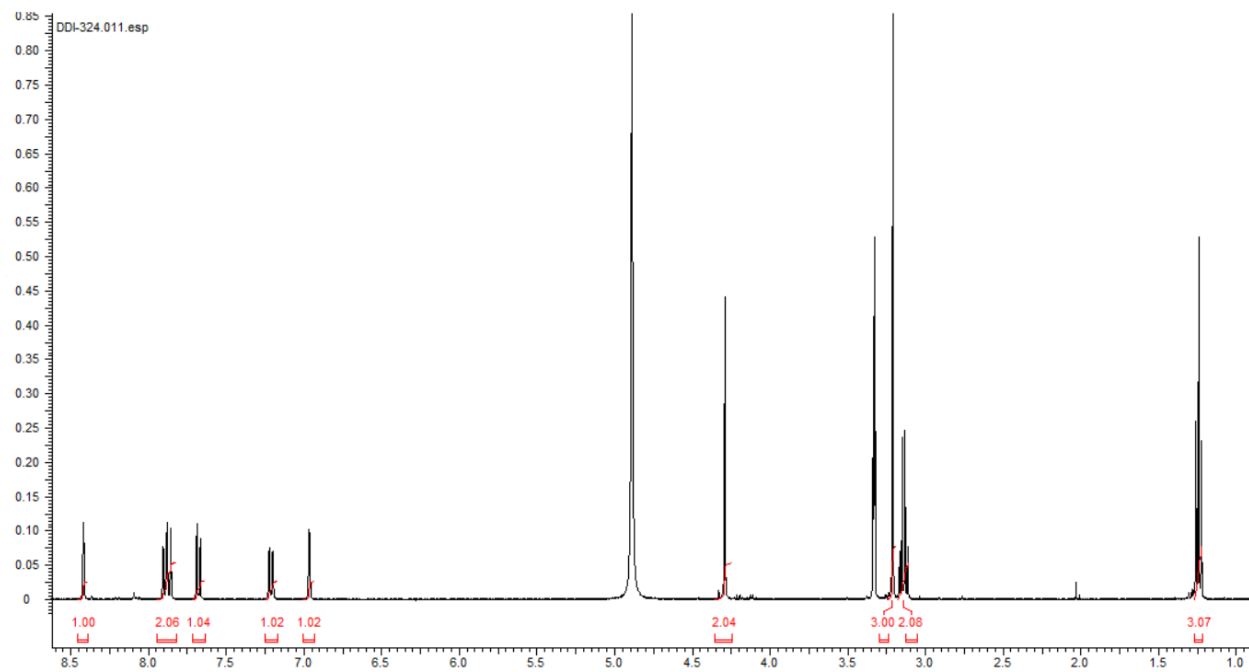


Figure S4. ^1H NMR spectrum of **3a** in CD_3OD .

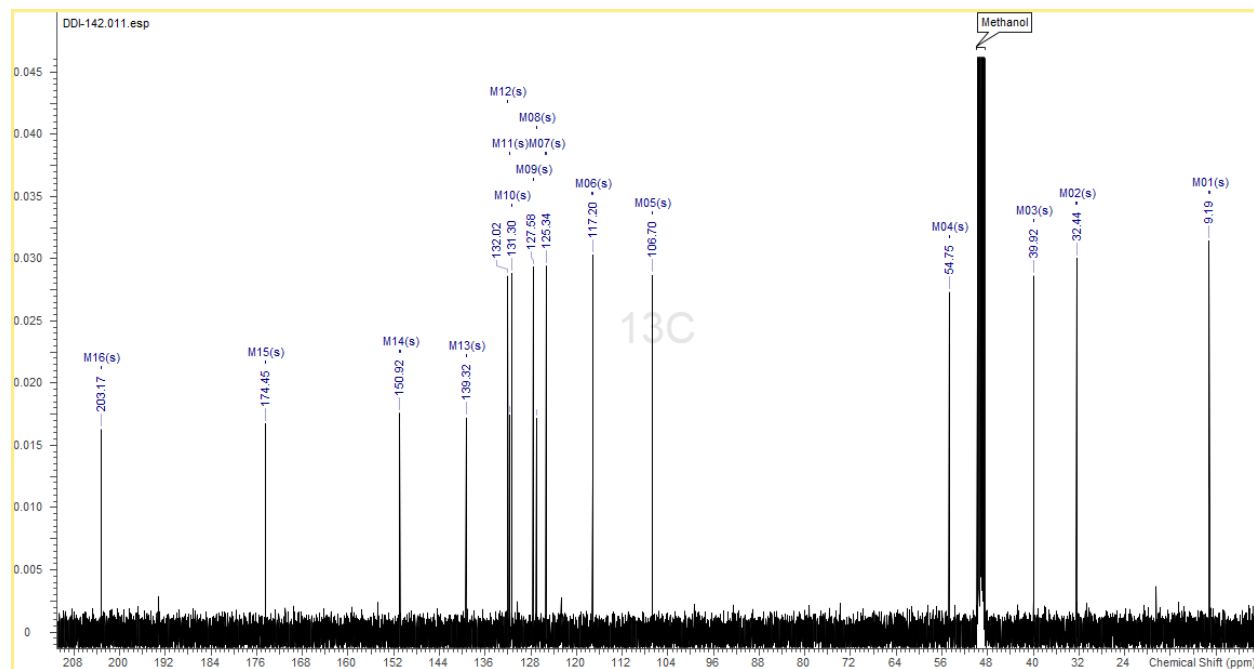


Figure S5. ^{13}C NMR spectrum of **3a** in CD_3OD .

Spectrum Source: Peak (1) in "+ BPC(all [-10]) Scan Sub"
Fragmentor Voltage: 120
Collision Energy: 0
Ionization Mode: ESI

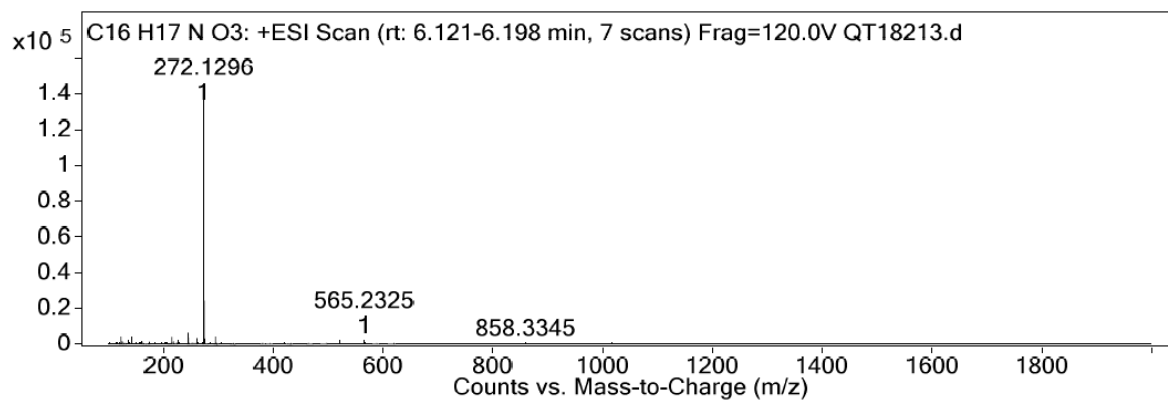


Figure S6. HRMS (ESI+) of 3a.

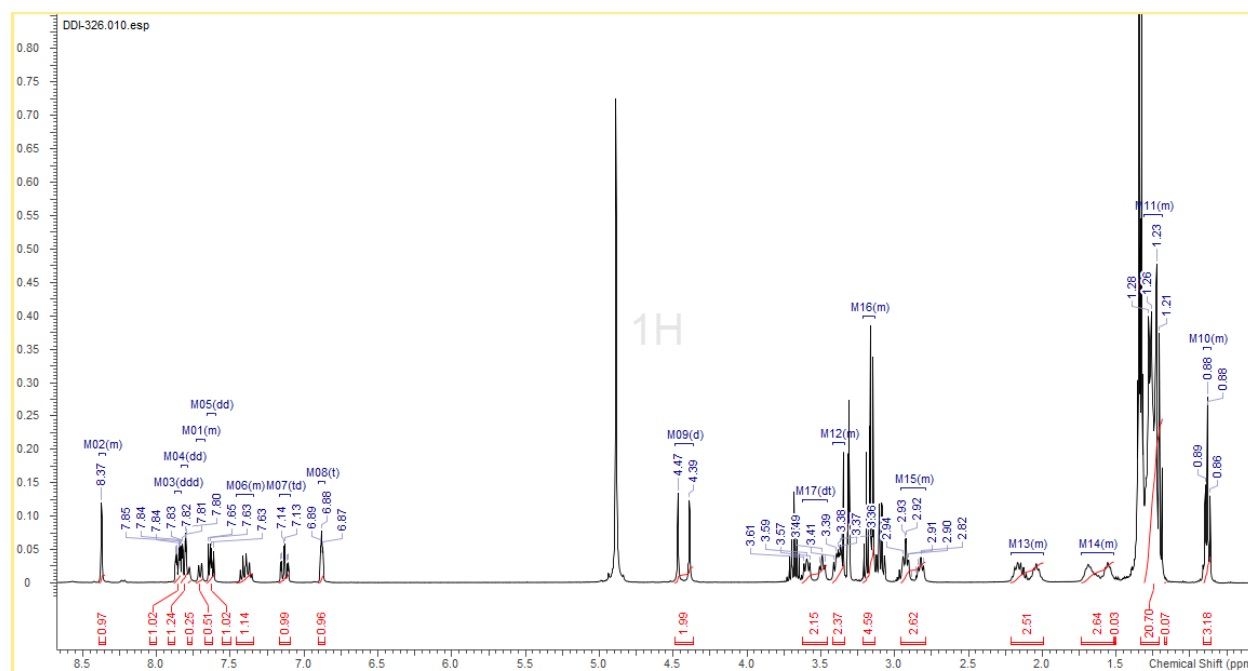


Figure S7. ¹H NMR spectrum of Pro12A in CD₃OD.

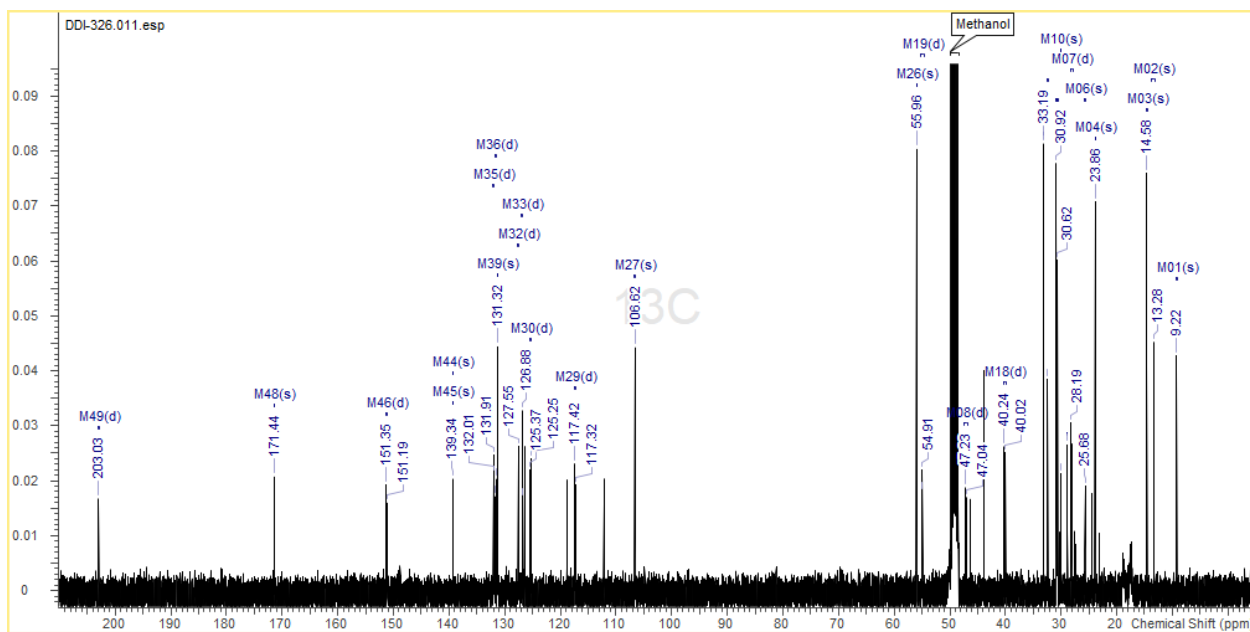


Figure S8. ^{13}C NMR spectrum of Pro12A in CD_3OD .

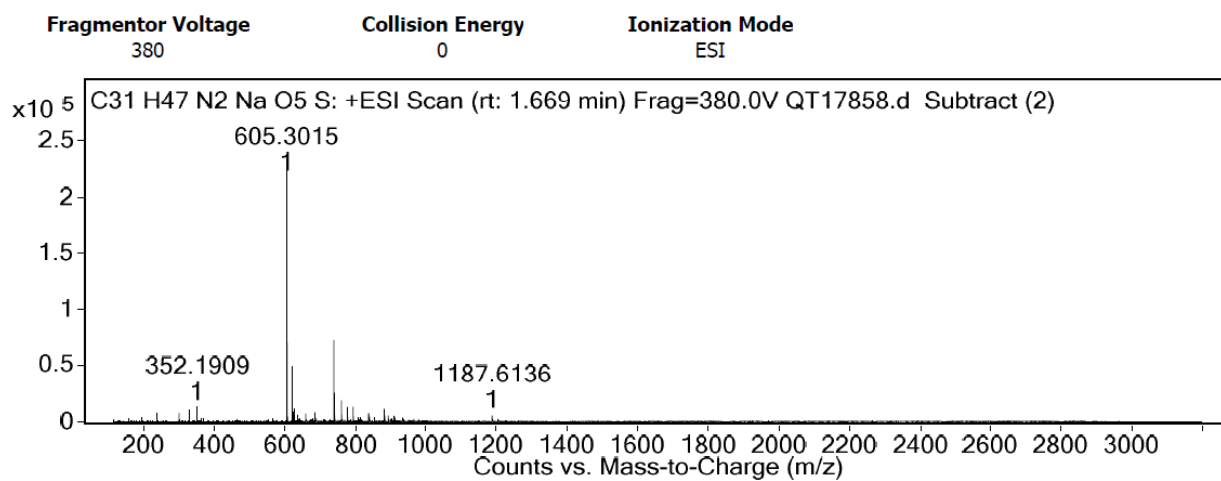


Figure S9. HRMS (ESI+) of Pro12A.

RESULTS

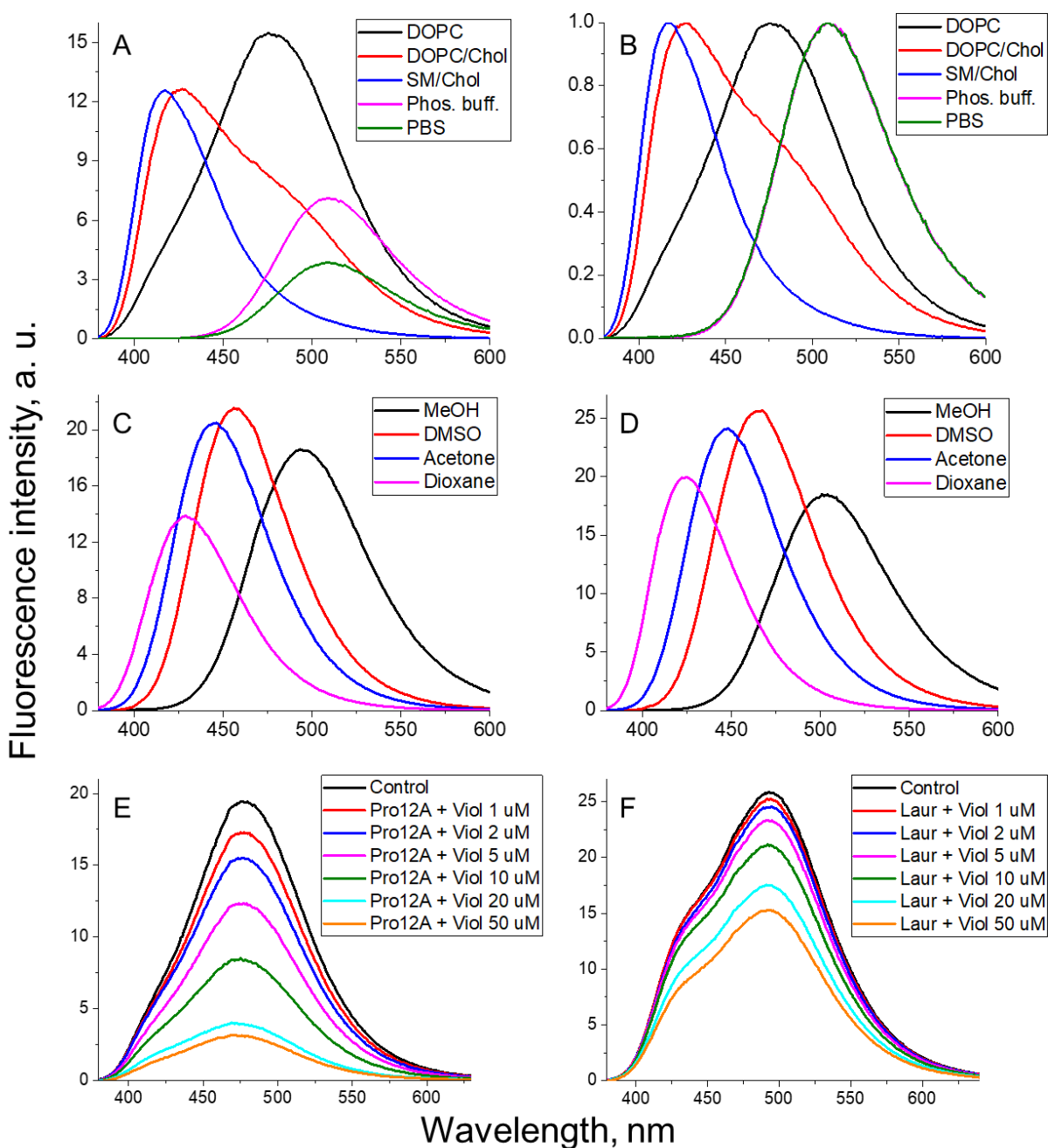


Figure S10. Fluorescence emission spectra of Pro12A in solvents and lipid vesicles of different composition: raw data (A) and normalized spectra (B); spectra of Pro12A (C) and Laurdan (D) in different solvents. Emission spectra of Pro12A (E) and Laurdan (F) in DOPC LUVs upon addition of different concentrations of dodecyl viologen. Probe concentration was 2 μM for (A-D) and 1 μM for (E-F). $\lambda_{\text{ex}} = 360$ nm in all cases. Total lipid concentration was 1 mM for all vesicles in (A-B) and 500 μM for (E-F).

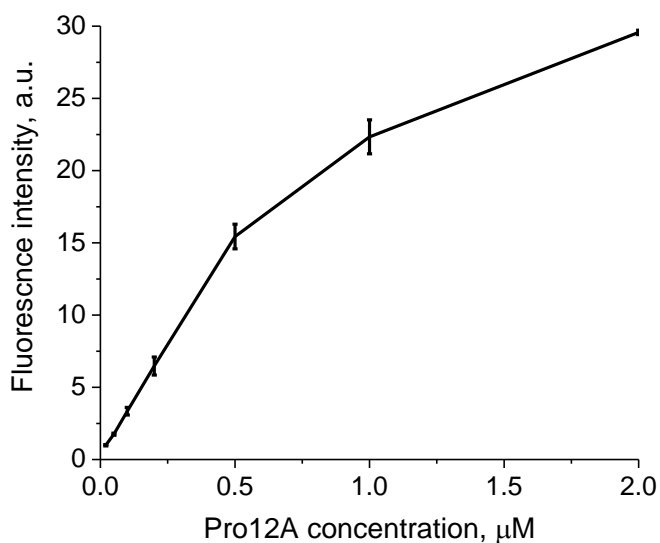


Figure S11. Fluorescence emission intensity of Pro12A at different concentrations in PBS. Excitation wavelength was 360 nm. The fluorescence intensity was recorded at the emission maximum. A deviation from the linear intensity increase is observed around 0.5 μM , indicating that around this concentration the dye starts forming micelles (critical micellar concentration), which leads to self-quenching.

Table S1. Fluorescent properties of probes in liposomes of different composition and organic solvents.^a

Solvent or medium	Probe					
	Pro12A			Laurdan		
	λ_{abs} , nm	λ_{em} , nm	QY, %	λ_{abs} , nm	λ_{em} , nm	QY, %
DOPC	372	476	54.4	356 ^c	488 ^c	25 ^c
DOPC/Chol	374	427	69.6	358 ^c	439 ^c	21 ^c
SM/Chol	379	417	51.5	357 ^c	435 ^c	19 ^c
PB ^b	371	509	25.2	-	-	-
PBS	369	509	12.5	-	-	-
Dioxane	349	429	42.9	348	425	38.8
Acetone	354	446	55.9	350	448	45.5
DMSO	359	456	54.5	359	467	67.9
MeOH	359	493	55.5	363	501	43.1

^a Probe concentrations were 2 μM . For liposomes total lipid concentration was 1 mM. Data for Laurdan in PB and PBS is not presented due to its insufficient solubility in aqueous media. Fluorescence quantum yields were measured using quinine sulfate in 0.5 M sulfuric acid ($\lambda_{\text{ex}}=360$ nm, $\text{QY}_{\text{ref}} = 54.6\%$)² as a reference.

^b PB – 20 mM phosphate buffer, pH 7.4.

^c Data taken from.³

REFERENCES

- (1) Grenier, M. C.; Davis, R. W.; Wilson-Henjum, K. L.; LaDow, J. E.; Black, J. W.; Caran, K. L.; Seifert, K.; Minbiole, K. P. The antibacterial activity of 4,4'-bipyridinium amphiphiles with conventional, bicephalic and gemini architectures. *Bioorg Med Chem Lett* **2012**, *22*, 4055-4058.
- (2) Brouwer, A. M. Standards for photoluminescence quantum yield measurements in solution (IUPAC Technical Report). *Pure Appl. Chem.* **2011**, *83*, 2213-2228.
- (3) Niko, Y.; Didier, P.; Mely, Y.; Konishi, G.; Klymchenko, A. S. Bright and photostable push-pull pyrene dye visualizes lipid order variation between plasma and intracellular membranes. *Sci Rep* **2016**, *6*, 18870.