Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Amphiphilic dynamic NDI and PDI probes: Imaging microdomains in giant unilamellar vesicles

David Alonso Doval, Andrea Fin, Miwa Takahashi-Umebayashi, Howard Riezman, Aurelien Roux, Naomi Sakai and Stefan Matile*

Department of Organic Chemistry, University of Geneva, Geneva, Switzerland, stefan.matile@unige.ch

Supplementary Information

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1. Materials and methods

As in refs. S1-S4, Supporting Information. Briefly, reagents for synthesis were purchased from Fluka and Aldrich, amino acid derivatives from Novabiochem and Bachem, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received. 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was from Sigma, and *p*-xylene-bis-pyridinium bromide (DPX) was from Invitrogen. Egg yolk phosphatidylcholine (EYPC), 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), Egg sphingomyelin (SM) and Cholesterol (CL) were purchased from Avanti Polar Lipids. Napthopyrene and Bodipy C5-HPC were obtained from Molecular Probes. ITO slides were purchased from Präzisions Glas & Optik GmbH (Iserlohn, Germany).

Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical (TLC) and preparative thin layer chromatography (PTLC) were performed on silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1 mm), respectively. HPLC was performed using Agilent 1100 series system. Melting points (Mp) were recorded on a heating table from Reichert (Austria). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, unless stated otherwise) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), b (broad). Microwave reactions were done using Initiator^{TM} Biotage laboratory microwave instrument. ^{1}H and ^{13}C NMR spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a

singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (*J*) given in Hz, or multiplet (m). ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). ESI-MS was performed on a Finnigan MAT SSQ 7000 instrument or a ESI API 150EX and are reported as mass-per-charge ratio *m/z* (intensity in %, [assignment]).

Vesicles were prepared with a Mini-Extruder from Avanti Polar Lipids (pore size 100 nm). Fluorescence measurements were performed with a FluoroMax-3 or a FluoroMax-4 spectrofluorometer (Horiba Scientific) equipped with a stirrer and a temperature controller. Fluorescence imaging was performed using a Zeiss LSM-710 confocal microscope.

Abbreviations. AcOH: Acetic acid; Calcd: Calculated; CL: Cholesterol; DCM: Dichloromethane; DMAc: N,N-Dimethyacetamide; DMF: Dimethylformamide; DMSO: Dimethylsulfoxide; DOPC: 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DPPC: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine; DPX: *p*-Xylene-bispyridinium bromide; EYPC: Egg yolk phosphatidylcholine; GUVs : Giant unilamellar vesicles; HPLC: High performance liquid chromatography; HPTS: 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt; *i*-PrOH: 2-Propanol; ITO: Indium tin oxide; LUVs: Large unilamellar vesicles; MeOH: Methanol; NDI: 1,4,5,8-Naphthalenetetracarboxylicdiimide; NH_4OAc: Ammonium acetate; PDI: 3,4,9,10 Perylenetetracarboxylicdiimide; rt: Room temperature; SM: Egg sphingomyelin; TEA: Triethylamine; TFA: Trifluoroacetic acid; THF: Tetrahydrofuran; Tris: Tris(hydroxymethyl)aminomethane.

2. Synthesis

2.1. Synthesis of cNDIs

Compound 10. This compound was prepared following previously reported procedures in ref. S1.

Compound 12. The tetraester **10** (50.0 mg, 0.099 mmol) was suspended in KOH/*i*-PrOH solution (1 M, 10 ml) and stirred at reflux for 15 h. The reaction mixture was then evaporated to dryness and then dissolved in 1:1 AcOH/H_2O mixture at pH = 4 (20 ml). To the homogeneous solution, the amine **11** (12.8 mg, 0.099 mmol) was added and the reaction mixture was heated under microwave irradiation at 160 °C for 5 min. NH_4OAc (15.3 mg, 0.198 mmol) was then added and the reaction mixture was heated under microwave irradiation at 160 °C for 30 minutes. The reaction mixture was then diluted with DCM (20 ml) and washed with 1 M $KHSO_4$ (2 × 50 ml), H_2O (50 ml) and brine (50 ml), dried over Na_2SO_4 and evaporated to dryness. Purification by PTLC (DCM/MeOH 20:1) afforded the NDI **12** (6.9 mg, 15%) as a yellow solid. R_f (DCM/MeOH 20:1): 0.45; Mp: >220 °C; IR (neat): 3226 (w), 2961 (w), 2923 (m), 2854 (w), 1700 (s), 1660 (s), 1575 (m), 1496 (w), 1446 (m), 1411 (w), 1379 (m), 1364 (w), 1325 (w), 1259 (m), 1236 (m), 1196 (m), 1103 (m), 1085 (m), 1073 (m), 1020 (s), 897 (w), 872 (w), 798 (s), 764 (m), 660 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 8.51 (s, 1H), 8.49 (s, 1H), 8.47 (s, 1H), 5.54 (q, 4H), 4.20-4.08 (m, 2H), 2.02-1.94 (m, 1H), 1.69-1.63 (m, 6H), 1.44-1.27 (m, 8H), 0.97-0.89 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 163.0 (s), 162.3 (s), 162.1 (s), 161.6 (s), 161.1 (s), 160.5 (s), 160.1 (s), 128.2 (s), 127.2 (s), 125.2 (s), 124.2 (s), 120.0 (d), 119.7 (d), 66.6 (t), 66.5 (t), 44.7 (t), 37.9 (q), 30.9 (t), 29.9 (s), 28.8 (t), 24.2 (t), 23.3 (t), 15.1 (q), 15.0 (q), 14.3 (q), 10.8 (q); MS (ESI, +ve):

467 (100, $[M + H]^+$), 933 (15, $[2M + H]^+$).

Compound 14. The imide **12** (20 mg, 0.042 mmol) was suspended in a 1:1 mixture of DCM and the amine **13** (5 ml). After stirring for 22 h at rt, the reaction mixture was evaporated to dryness and the residue was purified using PTLC (DCM/MeOH 98:2) to afford a regioisomeric mixture (2.5:1) of the imide **14** (15 mg, 72%) as a pink solid. R_f (DCM/MeOH 20:1): 0.52; Mp: >220 °C; IR (neat): 3180 (w), 2957 (w), 2926 (m), 2855 (w), 1702 (m), 1681 (m), 1641 (s), 1613 (w), 1584 (s), 1524 (w), 1494 (w), 1453 (m), 1380 (w), 1310 (m), 1291 (m), 1259 (s), 1200 (w), 1172 (w), 1142 (w), 1077 (m), 1063 (m), 1022 (s), 891 (w), 875 (w), 791 (s), 747 (w), 654 (w); ^{1}H NMR (500 MHz, $CDCl_3$, N/N: regioisomers): 9.85/9.57 (d, ^3J (H,H) = 7.8 Hz, 1H), 8.51/8.41 (s, 1H), 8.37/8.31 (s, 1H), 8.28/8.26 (s, 1H), 4.45 (q, ^3J (H,H) = 7.0 Hz, 2H), 4.20-4.06 (m, 3H), 1.99-1.91 (m, 1H), 1.63/1.61 (t, ^3J (H,H) = 7.0 Hz, 3H), 1.46-1.23 (m, 14H), 0.96-0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) *Major isomer:* 166.5 (s), 163.2 (s), 162.7 (s), 161.5 (s), 158.2 (s), 149.9 (s), 127.3 (s), 125.9 (s), 124.7 (s), 122.6 (s), 120.7 (d), 118.4 (d), 111.9 (s), 100.5 (s), 66.1 (t), 44.7 (d), 44.4 (t), 38.0 (d), 30.9 (t), 28.8 (t), 24.2 (t), 23.3 (q), 23.3 (t), 14.9 (q), 14.3 (q), 10.8 (q); ^{13}C NMR (100 MHz, $CDCl_3$) *Minor isomer:* 165.9 (s), 163.3 (s), 162.6 (s), 161.9 (s), 158.0 (s), 150.3 (s), 128.2 (s), 125.8 (s), 125.1 (s), 121.9 (s), 121.0 (d), 118.0 (d), 113.1 (s), 99.5 (s), 66.2 (t), 44.7 (d), 44.7 (t), 37.9 (d), 30.8 (t), 28.8 (t), 24.2 (t), 23.3 (q), 23.3 (t), 15.0 (q), 14.3 (q), 10.8 (q); MS (ESI, +ve): 480 (100, $[M + H]^+$), 959 (39, $[2M + H]^+$).

Compound 8. Under an atmosphere of nitrogen, the imide **14** (41 mg, 0.08 mmol), the boronic acid **15** (39 mg, 0.25 mmol), $Cu(OAc)_2$ (31 mg, 0.17 mmol) and TEA (26 mg, 0.25 mmol) were suspended in DMAc (8 ml) containing 4 Å molecular sieves. The reaction

mixture was purged with oxygen and then stirred at 55 °C for 20 h. The reaction mixture was then evaporated to dryness and the residue was purified by PTLC (DCM/MeOH 50:1) to afford a regioisomeric mixture (2:1) of the aldehyde **8** (31 mg, 62%) as a pink solid. R_f (DCM/MeOH 25:1): 0.8; Mp: >220 °C; IR (neat): 2969 (w), 2934 (w), 2872 (w), 1696 (m), 1679 (m), 1639 (s), 1581 (s), 1520 (w), 1495 (w), 1450 (m), 1382 (w), 1296 (s), 1279 (s), 1216 (s), 1167 (m), 1107 (w), 1065 (w), 1024 (w), 947 (w), 893 (w), 808 (w), 786 (m), 765 (s), 747 (s), 656 (w); ^{1}H NMR (400 MHz, $CDCl_3$, N/N: regioisomers): 10.13/10.11 (s, 1H), 9.75/9.62 (d, ^3J (H,H) = 7.5 Hz, 1H), 8.22/8.20 (s, 1H), 8.19/8.18 (s, 1H), 8.12/8.08 (d, ^3J (H,H) = 8.5 Hz, 2H), 7.60/7.59 (d, ^3J (H,H) = 8.5 Hz, 2H), 4.37 (q, ^3J (H,H) = 7.0 Hz, 2H), 4.20-4.00 (m, 3H), 1.90/1.89 (sept, ^3J (H,H) = 6.1 Hz, 1H), 1.60/1.56 (t, ^3J (H,H) = 7.0 Hz, 3H), 1.43/1.39 (m, 14H), 0.96-0.84 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) *Major isomer:* 191.5 (d), 166.1 (s), 162.8 (s), 162.8 (s), 161.4 (s), 158.3 (s), 149.6 (s), 141.1 (s), 136.5 (s), 130.8 (d), 130.1 (d), 126.9 (s), 125.3 (s), 124.1 (s), 121.3 (s), 120.9 (d), 118.1 (d), 112.3 (s), 100.1 (s), 66.0 (t), 44.7 (d), 44.3 (t), 38.0 (d), 30.8 (t), 28.7 (t), 24.8 (t), 23.3 (d), 23.2 (t), 14.9 (q), 14.2 (q), 10.8 (q); ^{13}C NMR (100 MHz, $CDCl_3$) *Minor isomer:* 191.4 (d), 166.0 (s), 162.9 (s), 162.6 (s), 161.4 (s), 157.8 (s), 150.4 (s), 141.0 (s), 136.6 (s), 130.9 (d), 139.9 (d), 127.8 (s), 124.7 (s), 124.4 (s), 121.4 (s), 120.9 (d), 117.9 (d), 112.5 (s), 99.1 (s), 66.1 (t), 44.8 (d), 44.5 (t), 37.9 (d), 30.8 (t), 28.7 (t), 24.8 (t), 23.2 (d), 23.2 (t), 14.8 (q), 14.2 (q), 10.8 (q); MS (ESI, +ve): 584 (100, $[M + H]^+$), 1168 (18, $[2M + H]^+$).

2.2. Synthesis of cPDIs

Compound 24. This compound was prepared following previously reported procedures in ref. S2.

Compound 25. To a solution of NaH (24 mg, 1.00 mmol) in ethanol (10 ml), **24** (100 mg, 0.14 mmol) was added. The mixture was refluxed at 80 °C for 18 h. The solution was allowed to cool down to rt and the solvent was removed *in vacuo*. Silica gel column chromatography of the residue (DCM) gave **25** (40 mg, 45%) as a red powder. Mp: > 220 °C; R_f (DCM): 0.3; IR (neat): 2924 (w), 2854 (w), 1694 (s), 1644 (s), 1596 (w), 1414 (w), 1245 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 9.65 (d, ^3J (H,H) = 8.4 Hz, 2H), 8.56 (d, ^3J (H,H) = 8.4 Hz, 2H), 8.48 (s, 2H), 5.12-5.02 (m, 2H), 4.57 (q, ^3J (H,H) = 6.8 Hz, 4H), 2.65-2.54 (m, 4H); 1.97-1.89 (m, 6H), 1.83-1.76 (m, 4H), 1.73 (t, ^3J (H,H) = 6.8 Hz, 6H), 1.53-1.45 (m, 6H); MS (ESI, +ve, 10:1 DCM-MeOH): 643 (100, $[M+H]^+$), 561 (60, [M - Cyclohexylamine]$^+$).

Compound 26. To a solution of KOH (215 mg, 3.83 mmol) in *i*-PrOH (15 ml), **25** (45 mg, 0.07 mmol) was added. The mixture was refluxed at 85 °C for 4 h. The solution was poured in acetic acid (20 ml) and allowed to cool down to rt. The solvent was removed *in vacuo* overnight. The residue was suspended in DMAc (10 ml) and cyclohexylamine (10 ml, 0.09 mmol) was added. The mixture was refluxed at 120 °C for 3 h. The solvent was evaporated *in vacuo*. Silica gel column chromatography (97:3 DCM-MeOH) of the residue gave **26** (14 mg, 35%) as a dark violet powder. Mp: > 220 °C; R_f (97:3 DCM-MeOH): 0.4; IR (neat): 2933 (w), 2853 (w), 1770 (w), 1730 (w), 1696 (w), 1653 (w), 1598 (w), 1260 (w), 1020 (s); ^{1}H NMR (400 MHz, $CDCl_3$): 9.69 (d, ^3J (H,H) = 8.4 Hz, 1H), 9.68 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.61 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.60 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.51 (s, 1H), 8.47 (s, 1H), 5.12-5.00 (m, 1H), 4.60 (q, ^3J (H,H) = 6.8 Hz, 4H), 2.64-2.53 (m, 2H), 1.97-1.89 (m, 2H), 1.83-1.77 (m, 3H), 1.75 (t, ^3J (H,H) = 6.8 Hz, 6H), 1.53-1.46 (m, 3H); MS (ESI, +ve, 10:1 CH_2Cl_2-MeOH): 562 (100, $[M+H]^+$).

Compound 27. To a solution of **26** (33 mg, 0.06 mmol) in DMF (4 ml), urea (141 mg, 2.35 mmol) was added. The mixture was refluxed at 130 °C for 14 h. The solution was allowed to cool down to rt and the solvent was evaporated *in vacuo*. Silica gel column chromatography (99:1 DCM-MeOH) of the residue gave **27** (24 mg, 72%) as a dark red-violet powder. Mp: > 220 °C; R_f (99:1 DCM-MeOH): 0.2; IR (neat): 3342 (w), 2922 (s), 2852 (w), 1702 (w), 1462 (w), 1273 (w); ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD 4:1): 9.51 (d, ^3J (H,H) = 8.4 Hz, 1H), 9.50 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.43 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.39 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.33 (s, 1H), 8.32-8.31 (m, 1H), 8.27 (s, 1H), 4.99-4.93 (m, 1H), 4.47 (q, ^3J (H,H) = 6.8 Hz, 4H), 2.58-2.47 (m, 2H), 1.95-1.84 (m, 2H), 1.78-1.71 (m, 3H), 1.65 (t, ^3J (H,H) = 6.8 Hz, 6H), 1.54-1.50 (m, 3H); MS (ESI, +ve, 10:1 CH_2Cl_2-MeOH): 561 (100, $[M+H]^+$).

Compound 18. Under atmosphere of nitrogen, the imide **27** (24 mg, 0.04 mmol), the 4-formylphenylboronic acid (19.3 mg, 0.13 mmol), $Cu(OAc)_2$ (15.6 mg, 0.08 mmol) and TEA (18 µl, 0.13 mmol) were suspended in $CHCl_3$ (4 ml) containing 4 Å molecular sieves. The reaction mixture was purged with oxygen and then stirred at 40 °C for 16 h. The reaction mixture was then evaporated to dryness and the residue was purified by silica gel column chromatography (95:5 CH_2Cl_2-acetone) to give **18** (18 mg, 65%) as a dark red powder. Mp: > 220 °C; R_f (95:5 CH_2Cl_2-acetone): 0.5; IR (neat): 2922 (s), 2852 (w), 1691 (s), 1649 (s), 1596 (s), 1414 (w), 1335 (w), 1260 (s), 1017(s); ^{1}H NMR (400 MHz, $CDCl_3$): 10.18 (s, 1H), 9.71 (d, ^3J (H,H) = 8.4 Hz, 1H), 9.69 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.64 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.59 (d, ^3J (H,H) = 8.4 Hz, 1H), 8.52 (s, 1H), 8.48 (s, 1H), 8.12 (d, ^3J (H,H) = 8.4 Hz, 2H), 7.59 (d, ^3J (H,H) = 8.4 Hz, 2H), 5.09-5.00 (m, 1H),), 4.58 (q, ^3J (H,H) = 6.8 Hz, 4H), 2.64-2.54 (m, 2H), 1.98-1.90 (m, 2H), 1.84-1.77 (m, 3H), 1.74 (t, ^3J (H,H) = 6.8 Hz, 6H), 1.53-

1.49 (m, 3H); MS (ESI, +ve, 10:1 CH_2Cl_2-MeOH): 665 (100, $[M+H]^+$).

2.3. Synthesis of amphiphiles

Compound 16. This compound was prepared following previously reported procedures in ref. S3.

Compound 4. Compound **16** (1.7 mg, 0.008 mmol) and R-NDI **8** (3.2 mg, 0.005 mmol) were dissolved in dry DMSO (1 ml) under nitrogen atmosphere, containing 4 Å molecular sieves. After stirring the mixture for 1 h at 60 °C, the reaction mixture was filtered through a 0.45 μm Millipore filter, and purified by reversed phase HPLC to afford pure **4** (4.0 mg, quantitative) as a pink solid. HPLC t_R = 11.4 min, column YMC-Pack ODS-A, S-5 mm, 120A C-18 (250 mm / 10 mm), elution gradient: from 40% of A (THF + 0.1% TFA) and 60% of B (H_2O + 0.1% TFA) to 70% of A and 30% of B in 12 min, flow rate: 3 ml/min; MS (ESI, +ve): 697 (100, $[M + H]^+$).

Compound 2. This compound was prepared following previously reported procedures in ref. S3.

Compound 6. Compound **2** (2.4 mg, 0.007 mmol), stearaldehyde **19** (2 mg, 0.007 mmol) and R-NDI **8** (4.3 mg, 0.007 mmol) were dissolved in dry DMSO (2 ml) under nitrogen atmosphere, containing 4 Å molecular sieves. After stirring the mixture for 1 h at 60 °C, the reaction mixture was filtered through a 0.45 μm Millipore filter, and purified by reversed phase HPLC to afford pure **6** (2.4 mg, 30%) as a pink solid. HPLC t_R = 15.9 min, column

YMC-Pack ODS-A, S-5 mm, 120A C-18 (250 mm / 10 mm), elution gradient: from 40% of A (THF + 0.1% TFA) and 60% of B (H_2O + 0.1% TFA) to 70% of A and 30% of B in 12 min, flow rate: 3 ml/min; MS (ESI, +ve): 1034 (100, $[M + H]^+$).

Compound 7. Compound **16** (0.5 mg, 0.003 mmol) and PDI **18** (2 mg, 0.003 mmol) were dissolved in dry DMSO (1 ml) under nitrogen atmosphere, containing 4 Å molecular sieves. After stirring the mixture for 1 h at 60 °C, the reaction mixture was filtered through a 0.45 μm Millipore filter, and purified by reversed phase HPLC to afford pure **7** (2.4 mg, quantitative) as a pink solid. HPLC t_R = 11.7 min, column YMC-Pack ODS-A, S-5 mm, 120A C-18 (250 mm / 10 mm), elution gradient: from 40% of A (THF + 0.1% TFA) and 60% of B (H_2O + 0.1% TFA) to 70% of A and 30% of B in 12 min, flow rate: 3 ml/min; MS (ESI, +ve): 778 (100, $[M + H]^+$).

Compound 22. Compound **23** (0.6 mg, 0.001 mmol) and R-NDI **8** (2 mg, 0.003 mmol) were dissolved in dry DMSO (1 ml) under nitrogen atmosphere, containing 4 Å molecular sieves. After stirring the mixture for 1 h at 60 °C, the reaction mixture was filtered through a 0.45 μm Millipore filter, and purified by reversed phase HPLC to afford pure **22** (2.4 mg, quantitative) as a pink solid. HPLC t_R = 16.0 min, column YMC-Pack ODS-A, S-5 mm, 120A C-18 (250 mm / 10 mm), elution gradient: from 40% of A (THF + 0.1% TFA) and 60% of B (H_2O + 0.1% TFA) to 70% of A and 30% of B in 12 min, flow rate: 3 ml/min; MS (ESI, +ve): 2057 (100, $[M + H]^+$).

3. DNA activation

3.1. Vesicle preparation

Following the procedures in ref. S3, a thin lipid film was prepared by evaporating a solution of 25 mg EYPC in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (room temperature) and then *in vacuo* overnight. The resulting film was hydrated with 1.0 ml buffer (5 mM HPTS, 16.5 mM DPX, 10 mM Tris, 72 mM NaCl, pH 7.4) for more than 30 min, subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size: 100 nm). Extravesicular components were removed by gel filtration (Sephadex G-50) with 10 mM Tris, 107 mM NaCl, pH 7.4. Final conditions: ~5 mM EYPC; inside: 5 mM HPTS, 16.5 mM DPX, 10 mM Tris, 72 mM NaCl, pH 7.4; outside: 10 mM Tris, 107 mM NaCl, pH 7.4.

3.2. DNA activation

Adapting the general procedures in ref. S3, EYPC-LUV stock (5 μl) was diluted with a buffer (10 mM Tris, 107 mM NaCl, pH 7.4), placed in a thermostated fluorescence cuvette (25 ˚C) and gently stirred (total volume in the cuvette, ~2000 μl; final lipid concentration, ~13 μM). HPTS efflux was monitored at λ_{em} 511 nm (λ_{ex} 413 nm) as a function of time after addition of activator (hydrazones, 10 μl in DMSO) at t = 0 s, transporter (calf thymus DNA, 20 μl stock solution in buffer, 1.25 μg/ml final concentration) at t = 40 s and 1.2% aqueous triton X-100 (40 μl, 0.024% final concentration) at t = 200 s. Fluorescence intensities were normalized to fractional emission intensity I(t) using equation (*S1*)

$$I(t) = (I_t - I_0) / (I_\infty - I_0) \qquad (S1)$$

where $I_0 = I_t$ at DNA addition, $I_\infty = I_t$ at saturation after lysis. Effective concentration for hydrazone (EC_{50}) and Hill coefficient (n) were determined by plotting the fractional activity $Y = I(t)$ at saturation just before lysis, t = ~190 s as a function of hydrazone concentration $c_{hydrazone}$ and fitting them to the Hill equation (*S2*)

$$Y = Y_0 + (Y_{MAX} - Y_0) / \{1 + (EC_{50} / c_{hydrazone})^n\} \qquad (S2)$$

where Y_0 is Y without hydrazone, Y_{MAX} is Y with an excess hydrazone at saturation, EC_{50} is the concentration of hydrazone required to reach 50% activity and n is the Hill coefficient.

4. Partition coefficients

4.1. DOPC vesicles

A thin lipid film was prepared by evaporating a solution of 25 mg DOPC in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (rt) and then *in vacuo* overnight. The resulting film was hydrated with 2.0 ml buffer (10 mM Tris, 100 mM NaCl, pH 7.4) for more than 30 min (rt), subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size, 100 nm). Final conditions: ~32 mM DOPC; 10 mM Tris, 100 mM NaCl, pH 7.4.

4.2. DOPC/CL vesicles

A thin lipid film was prepared by evaporating a solution of 16.7 mg DOPC and 4.1 mg CL in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (rt) and then *in vacuo* overnight. The resulting film was hydrated with 2.0 ml buffer (10 mM Tris, 100 mM NaCl, pH 7.4) for more than 30 min (rt), subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size : 100 nm). Final conditions: ~21 mM DOPC, ~10 mM CL (total lipid ~31 mM), 10 mM Tris, 100 mM NaCl, pH 7.4.

4.3. DPPC vesicles

A thin lipid film was prepared by evaporating a solution of 22 mg DPPC in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (at 50 °C) and then *in vacuo* overnight. The resulting film was hydrated with 2.0 ml buffer (10 mM Tris, 100 mM NaCl, pH 7.4) for more than 30 min (at 50 °C), subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size : 100 nm). Final conditions: ~30 mM DPPC, 10 mM Tris, 100 mM NaCl, pH 7.4.

4.4. DPPC/CL vesicles

A thin lipid film was prepared by evaporating a solution of 17 mg DPPC and 4.5 mg CL in 1 ml MeOH/$CHCl_3$ (1:1) on a rotary evaporator (at 50 °C) and then *in vacuo* overnight. The resulting film was hydrated with 2.0 ml buffer (10 mM Tris, 100 mM NaCl, pH 7.4) for more

than 30 min (at 50 °C), subjected to freeze-thaw cycles (5×) and extrusions (15×) through a polycarbonate membrane (pore size : 100 nm). Final conditions: ~23 mM DPPC, ~11 mM CL (total lipid ~34 mM), 10 mM Tris, 100 mM NaCl, pH 7.4.

4.5. Partition coefficients

LUVs composed of DOPC, DOPC/CL, DPPC or DPPC/CL (0.1-10.0 mM) were added to a solution of **4** (10 μM), or **6** (10 μM) or **7** (7 μM), maintained at 25 °C with continuous stirring in a total volume of 2 ml buffer solution (10 mM Tris, 100 mM NaCl, pH 7.4). The fluorescence spectra were recorded at equilibrium after addition of lipids (2 h for DPPC or DPPC/CL LUVs and 1 h for DOPC or DOPC/CL LUVs). Fluorescence spectra for **4** and **6** were recorded using an excitation wavelength of 540 nm. Fluorescence spectra of **7** were recorded using an excitation wavelength of 570 nm. K_x was calculated based on the fits of the following equation (*S3*) to the data:

$$I = I_{MIN} + (I_{MAX} - I_{MIN}) / (1 + [H_2O] / K_x [Lipid]) \qquad (S3)$$

where I is the change in fluorescence intensity (at 600 nm for compound **7** or at 575 nm for compounds **4** and **6**) for a given concentration of lipid, I_{MAX} is the maximum fluorescence at high lipid concentrations, I_{MIN} is the fluorescence without lipids, [Lipid] is the lipid concentration, and $[H_2O]$ is the molar concentration of water (55.3 M).

5. Microdomains in GUVs

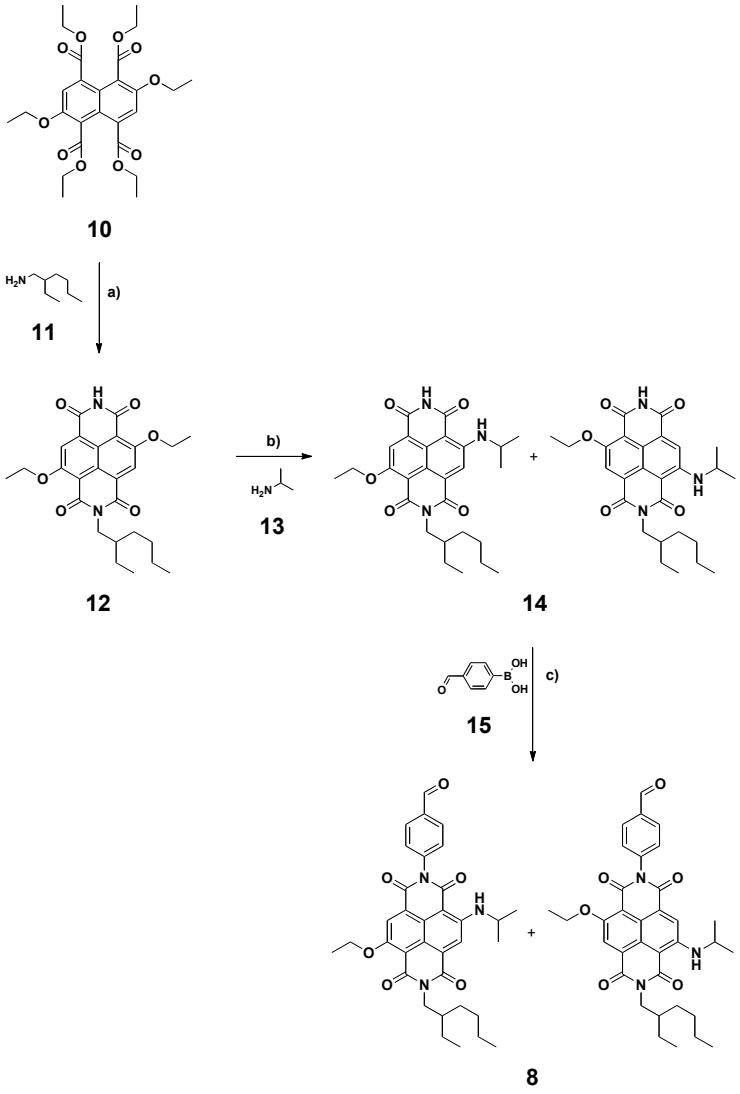
5.1. Prepartion of GUVs

Adapting the procedures in ref. S4, GUVs were prepared by electroformation method. Briefly, the ternary mixture of DOPC, SM and CL (0.24 : 0.56 : 0.2) was dissolved in $CHCl_3$ with 0.5 or 0.1 mol% of amphiphiles **4**, **5**, **6**, **7** or **22** and 0.05 mol% of the Lo marker Naphthopyrene or the Ld marker Bodipy C5-HPC. The mixture was deposited on two indium tin oxide (ITO) slides and the solvent was evaporated *in vacuo* for 1 h at 50 °C. The resulting film was hydrated with 1.0 ml buffer (100 mM sucrose) and electroswelling was performed at 60 °C; voltage amplitude: 1.2 V, frequency: 10 Hz, 2 h.

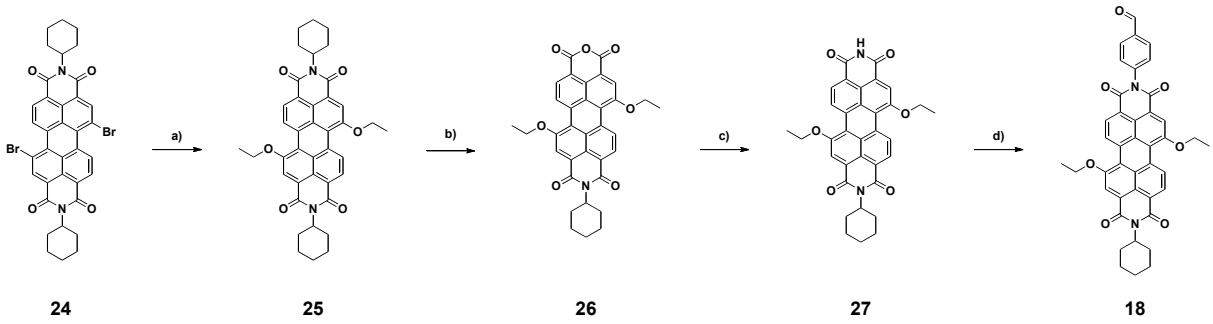
5.2. Fluorescence microscopy

Fluorescence images were obtained using a Zeiss-LSM 710 confocal microscope equipped with a 63X oil immersion objective (NA 1.4). The excitation wavelengths were 543 nm for compounds **4**, **5**, **6**, **7** or **22**, 405 nm and 488 nm for naphthopyrene and Bodipy C5-HPC, respectively.

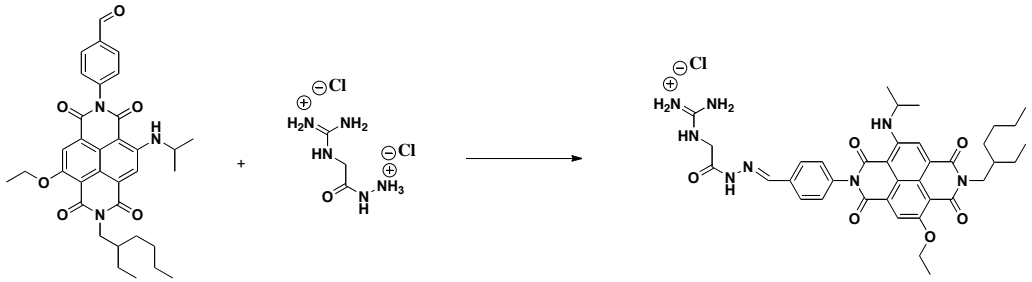
6. Supporting schemes & figures



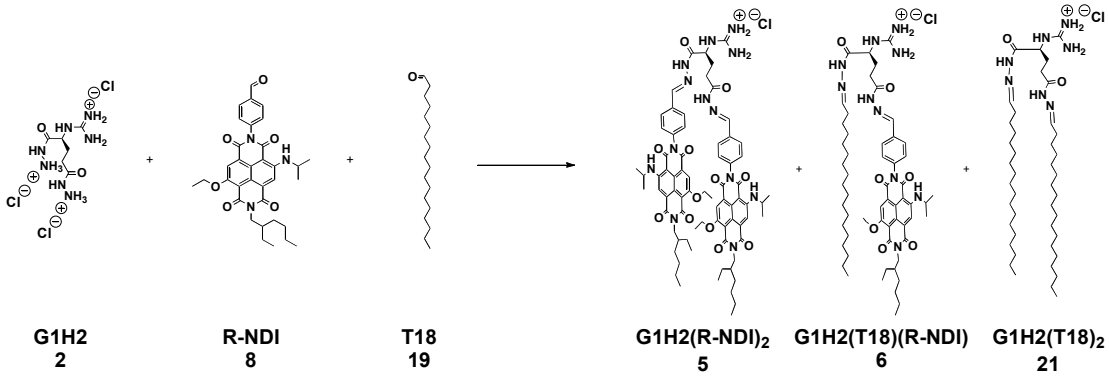
Scheme S1. Synthesis of compound **8**. a) 1. KOH/*i*-PrOH, reflux, 15 h, 2. **11**, AcOH/H_2O (1:1), pH = 4, 160 °C, 5 min μW, 3. NH_4OAc, AcOH/H_2O (1:1), 160 °C, 30 min μW, 15%; b) *i*-propylamine (**13**)/DCM (1:1), rt, 22 h, 72%; c) $Cu(OAc)_2$, TEA, 4-formylphenylboronic acid (**15**), DMAc, O_2, 55 °C, 24 h, 62%.



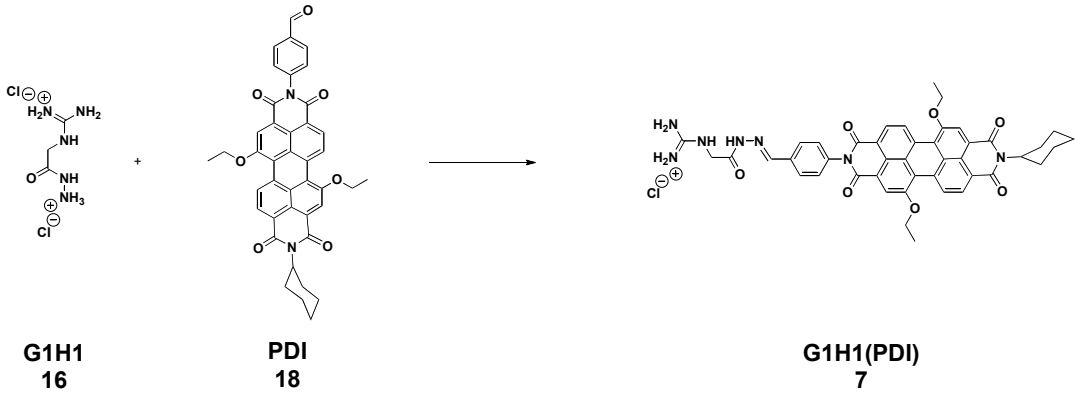
Scheme S2. Synthesis of compound **18**. a) Ethanol, NaH, reflux, 18 h, 45%; b) 1. *i*-PrOH, KOH, reflux, 4 h; 2. DMAc, Cyclohexylamine, 120 °C, 3 h, 35%; c) DMF, Urea, 130 °C, 14 h, 72%; d) $Cu(OAc)_2$, TEA, 4-formylphenylboronic acid, $CHCl_3$, O_2, 40 °C, 16 h, 65%.



Scheme S3. Synthesis of **4**. DMSO, 60 °C, 1 h, quantitative.



Scheme S4. Synthesis of **6**. DMSO, 60 °C, 1 h, 30% (**6**).



Scheme S5. Synthesis of **7**. DMSO, 60 °C, 1 h, quantitative.

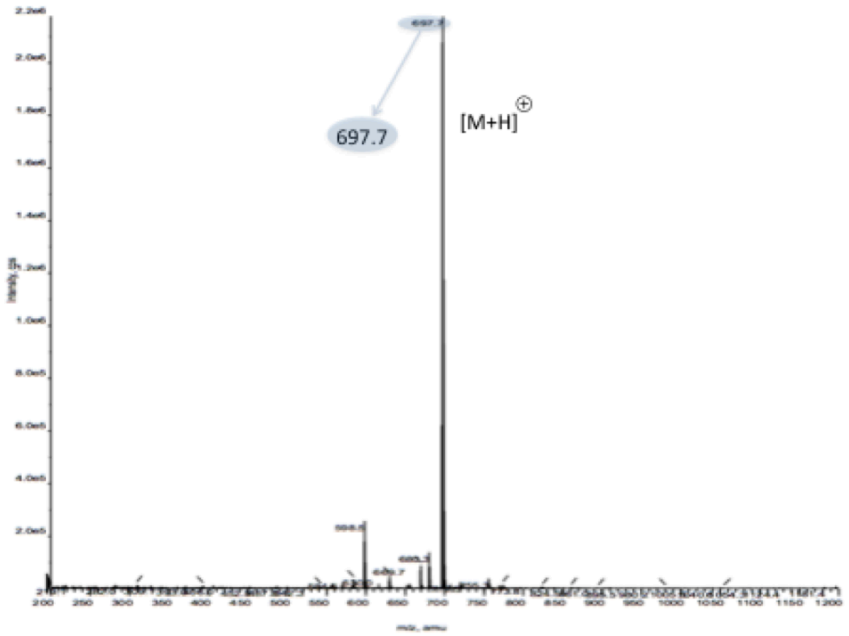


Fig. S1. ESI MS spectrum of **4**.

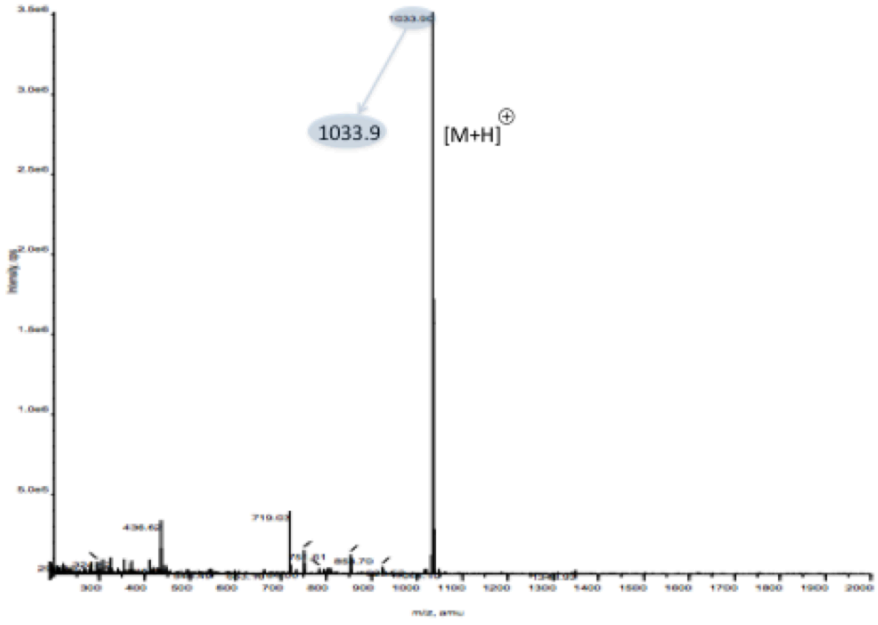


Fig. S2. ESI MS spectrum of **6**.

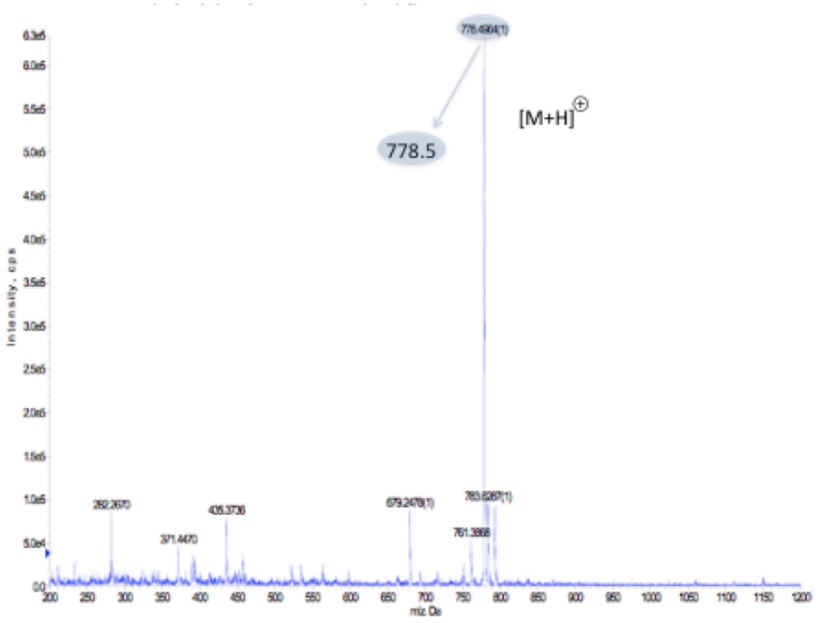


Fig. S3. ESI MS spectrum of **7**.

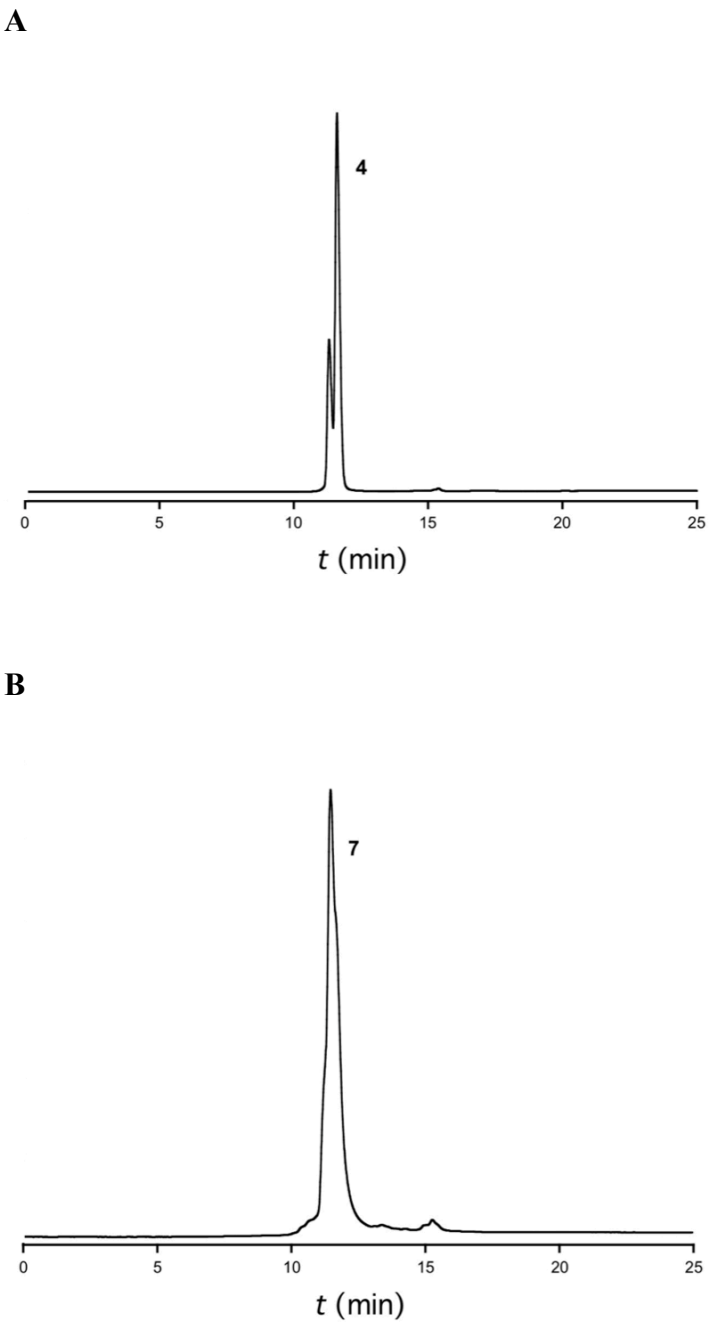


Fig. S4. (**A**) RP-HPLC chromatograms of the cNDI amphiphle **4** (t_R = 11.4 min) and (B) cPDI amphiphile **7** (t_R = 11.7 min) with detection at 540 nm; HPLC column YMC-Pack ODS-A, S-5 mm, 120A C-18 (250 mm / 10 mm), elution gradient: from 40% of A (THF + 0.1% TFA) and 60% of B (H_2O + 0.1% TFA) to 70% of A and 30% of B in 12 min, flow rate: 3 ml/min.

A **B**

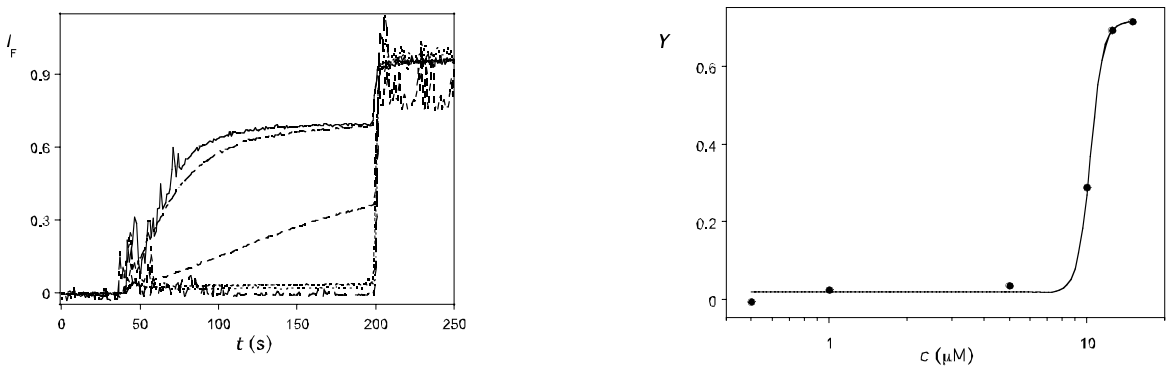


Fig. S5. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at t = 40 s and triton X-100 (0.024% final concentration) at t = 200 s, demonstrating increasing transporter activity with increasing concentrations of **4** (0.5-15 μM). (**B**) Representative dose-response curve for DNA activation with **4** obtained by plotting the fractional activity Y as a function of **4** concentration, giving EC_{50} for **4** of 10.3 ± 0.1 μM, Y_{MAX} of 71.6 ± 2.4% and Hill coefficient n of 17.1 ± 1.2.

A **B**

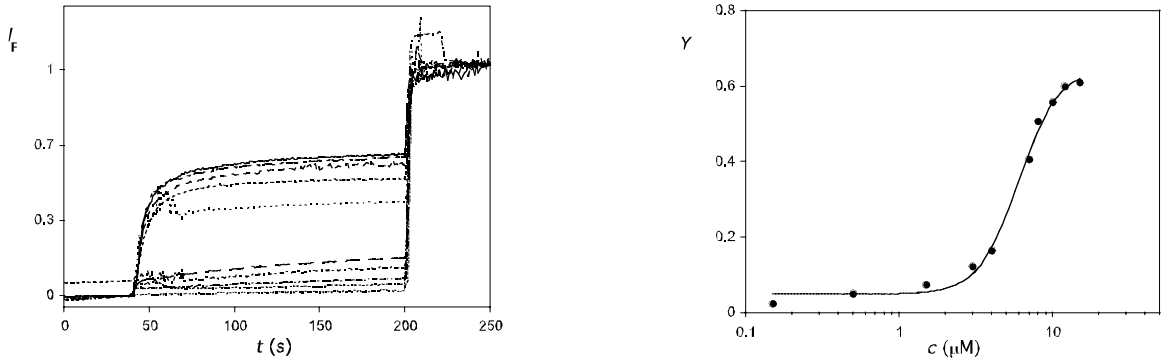


Fig. S6. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at t = 40 s and triton X-100 (0.024% final concentration) at t = 200 s, demonstrating increasing transporter activity with increasing concentrations of **6** (0.15-15 μM). (**B**) Representative dose-response curve for DNA activation with **6** obtained by plotting the fractional activity Y as a function of **6** concentration, giving EC_{50} for **6** of 6.2 ± 0.3 μM, Y_{MAX} of 66.0 ± 3.0% and Hill coefficient n of 3.1 ± 0.4.

A **B**

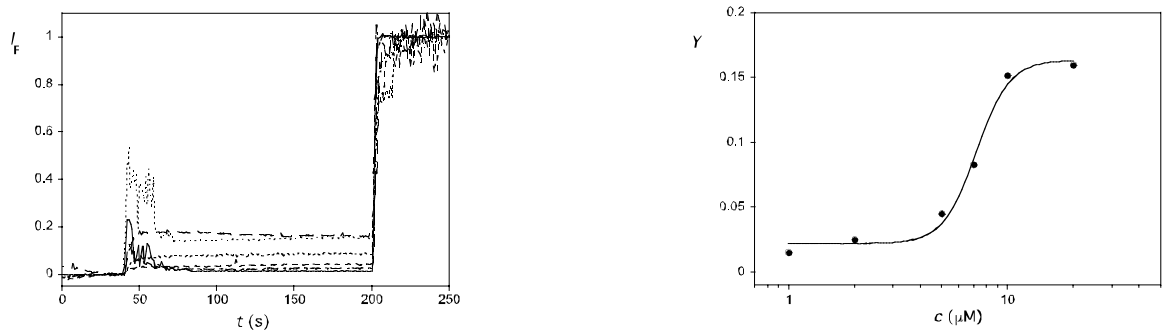


Fig. S7. (**A**) Representative normalized kinetics of transporter-mediated increase in HPTS fluorescence, following addition of transporter (calf thymus DNA, 1.25 μg/ml final concentration) at t = 40 s and triton X-100 (0.024% final concentration) at t = 200 s, demonstrating increasing transporter activity with increasing concentrations of **7** (1-20 μM). (**B**) Representative dose-response curve for DNA activation with **7** obtained by plotting the fractional activity Y as a function of **7** concentration, giving EC_{50} for **7** of 7.2 ± 0.4 μM, Y_{MAX} of 16.3 ± 0.9% and Hill coefficient n of 5.7 ± 1.6.

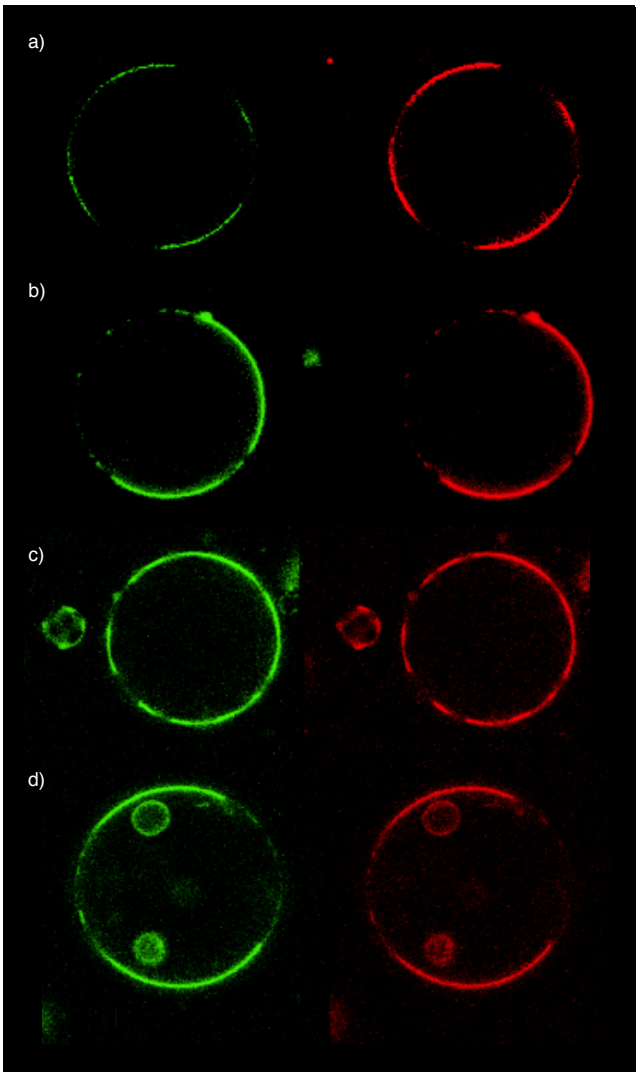


Fig. S8. Gallery of fluorescent images of GUVs composed of SM/DOPC/CL 56:24:20 with 0.5 or 0.1 mol% (a) **4**, (b) **5,** (c) **6** and (d) **22** (red emission, λ_{ex} 543 nm) and 0.05 mol% BODIPY FL C5-HPC in the Ld phase (green emission, λ_{ex} 488 nm). Single plane images of the equator region show simultaneously recorded red (right) and green emission separately (left). The diameters of all shown GUVs were around ~10 μm, their dispersity.

7. Supporting References

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S4. N. F. Morales-Penningston, J. Wu, E. R. Farkas, S. L. Goh, T. M. Konyakhina, J. Y. Zheng, W. W. Webb and G. W. Feigenson, *Biochim. Biophys. Acta*, 2010, **1798**, 1324–1332.