

Supplementary Information for “Aplosspan:” A Membrane-spanning, Ion-selective Channel that Functions in Phospholipid Bilayers

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Carboxyfluorescein (CF) release:

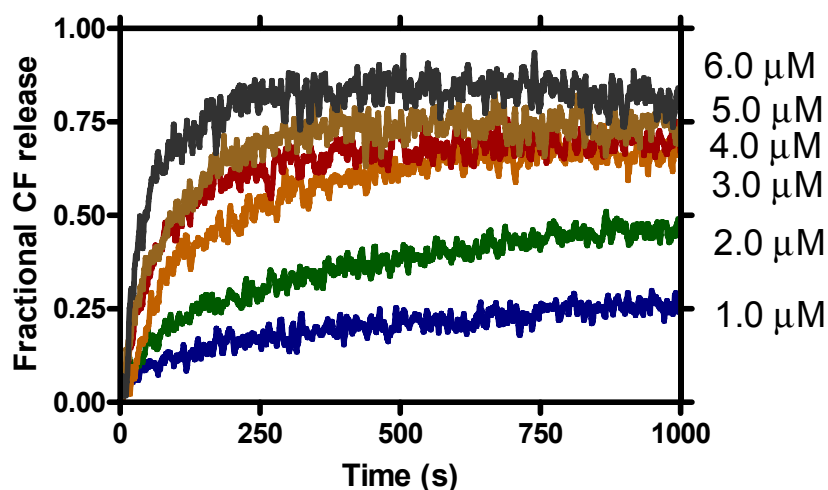


Figure S1

Caption: Carboxyfluorescein release from liposomes (DOPC, 10 mM) mediated by **1**. Internal buffer (20 mM CF, 100 mM KCl, 10 mM HEPES, pH = 7.0), External buffer (100 mM KCl, 10 mM HEPES, pH = 7.0); stocking solution: 1 mM of **1** in DMSO

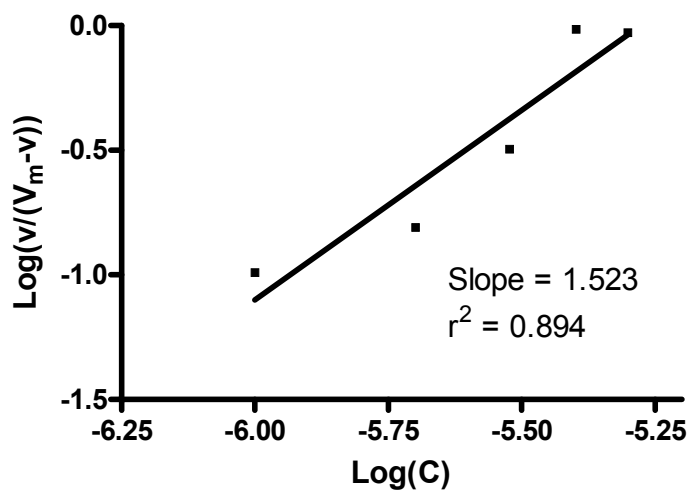
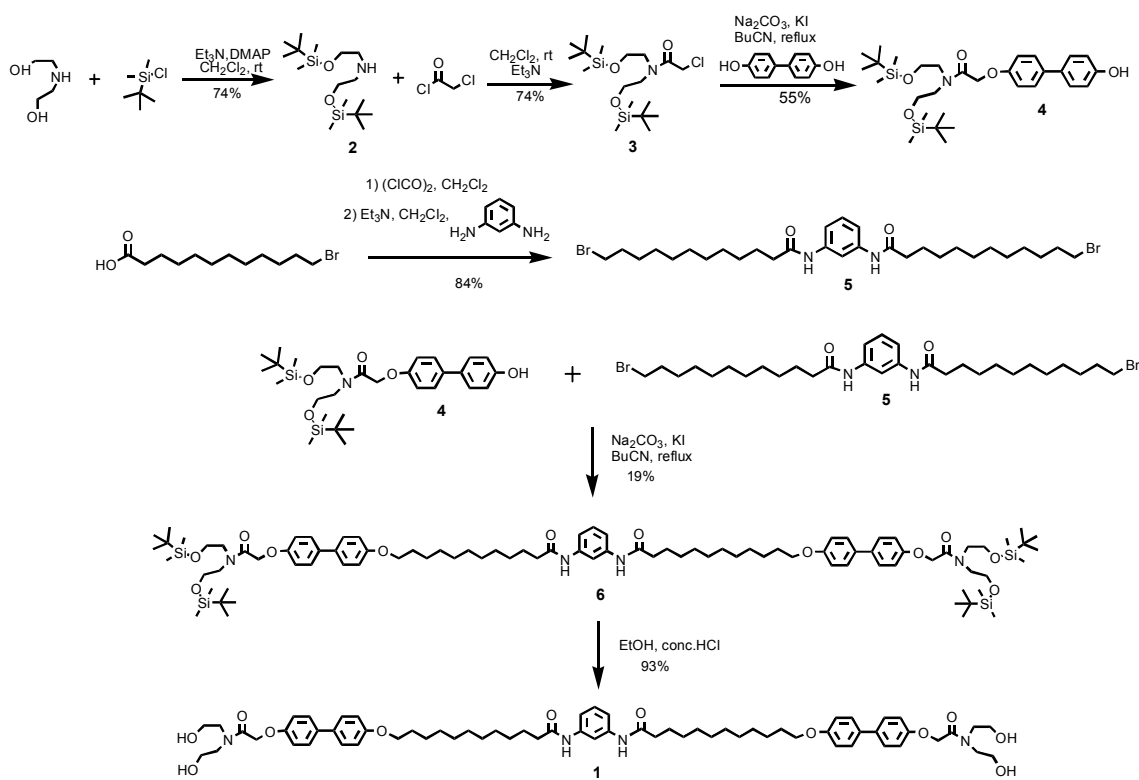


Figure S2

Caption: Hill plot of compound 1



Scheme S1

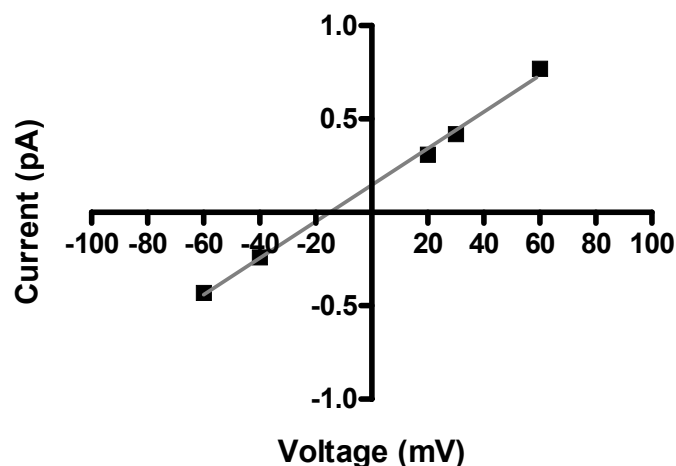


Figure S3

Caption: Cation *vs* Anion selectivity experiment by BLM in asymmetric buffer solutions (*trans*: 450 mM KCl, 10 mM HEPES, pH = 7; *cis*: 3 M KCl, 10 mM HEPES, pH = 7).

General: ^1H -MNR and ^{13}C -NMR spectrum were recorded on a Gemini 300 spectrometer at 300 MHz and 75 MHz and were reported in ppm (δ) down field from internal $(\text{CH}_3)_4\text{Si}$. Melting points were determined on a Thomas Hoover apparatus in open capillaries. Thin layer chromatography analyzes were performed on silica gel 60-F-254 with a 0.2 mm thickness. Preparative chromatography columns were packed with silica gel (Kieselgel 60, 70-230 mesh or Merck grade 9385, 230-240 mesh, 60 Å). All reactions were conducted under dry N_2 unless otherwise stated. All reagents were the best (non-LC) grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL Mstation (JMS-700) mass spectrometer (UMSL).

Sodium release experiments: Vesicles were prepared using the reverse phase procedure of Szoka and Papahadjopoulos. Vesicle comprised of 1,2-dioleoyl-3-phosphocholine (DOPC). Internal buffer was made by 750 mM NaCl/15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH was 7.0 which was adjusted with $(\text{CH}_3)_3\text{NOH}$. Ion exchange column was a Sephadex G25 column. The vesicle size was measured by a Brookhaven ZetaPALS zeta potential analyzer. All data were collected by Axoscope 7.0 using a Digidata 1322 series interface. Sodium transport was measured using a Micro-Combination pH/sodium electrode in aqueous sodium-free buffer (750 mM cholineCl/15 mM HEPES, pH = 7.0). The lipids concentration is 0.4 mM and the system has a total volume of 2 mL. The channel in DMSO was add in the system and measured for

25 minutes. At the end of the measurement, addition of 200 L 10% aqueous-*n*-octyl glucoside induced complete lysis of the vesicles to achieve total sodium release. All data was analyzed using OriginPro 7.

Carboxyfluorescein (CF) dequenching from unilamellar liposomes: Lipids (15 mg) 1,2-dioleoyl-3-phosphocholine (DOPC) (Avanti Polar Lipids,AL) was dissolved in 0.35 ml of Et₂O. To this was added 0.35 ml of 20 mM carboxyfluorescein in 100 mM KCl: 10 mM HEPES and the final pH were adjusted to 7.0 with KOH. This mixture was sonicated at 1200 W for 3x 20 s at 20 °C to produce a stable emulsion. The Et₂O was removed (water aspirator, 30 °C) in a round bottom flask rotating at 40 rpm. The 0.5 ml suspension was supplemented with an additional 0.35 mL of 20 mM carboxyfluorescein solution. This mixture was passed (five times) through a 200 nm nucleopore filter. The extra-vesicular carboxyfluorescein was removed by passing the liposome-dye mixture over a 1x20 cm Sephacryl G-25 column (Sigma) in 100 mM KCl: 10 mM HEPES (pH = 7.0). The liposome peak was collected and analyzed by dynamic light scattering with a mean diameter around 200 nm. Prior to use, carboxyfluorescein-containing vesicles were diluted to desired concentration (10 μM) in a reaction volume of 2 mL. The fluorescence (excitation 497 nm: emission 520 nm at 2 nm bandpass) was monitored at 25 °C. Compounds were added as a 1.0 mM solution in DMSO to the indicated concentrations. Dequenching, F_{520} , was computed as the fraction of total release upon addition of 1% Triton X100:

$$F_{520} = (F - F_0) / (F_{\text{Triton}} - F_0)$$

Where F_0 and F_{Triton} being the zero time and the fluorescence produced in the presence of Triton X-100. Dequenching data were fit using a nonlinear least squares method based upon the Levenberg–Marquardt algorithm.

Planar bilayer clamp experiment. All BLM experiments were performed by using Planar Lipid Bilayer Workstation from “Warner Instruments”. Planar bilayer membranes were formed across an aperture in a delrin barrier by painting lipids (Asolectin, extract from soybeans, 25 mg/mL in *n*-decane) over the 200 μm aperture on the side of a cuvette fitted into a chamber. Cuvette (*trans*) and chamber (*cis*) contained symmetric buffer solutions (450 mM KCl, 10 mM HEPES, pH = 7) or asymmetric buffer solutions (*trans*: 450 mM KCl, 10 mM HEPES, pH = 7; *cis*: 3 M KCl, 10 mM HEPES, pH = 7) when selectivity experiment was performed. Membrane formation was confirmed by the capacitance reading on Bilayer Clamp Amplifier (membranes with a capacitance lower than 100 pF were discarded). An aliquot of a trifluoroethanol solution of the compound (concentration varies depending on the feature of compounds) was stirred into the buffer on the chamber side (“*cis*” side where the input electrode is immersed) to achieve the desired concentration (1 μM). Records were filtered with a 4-pole Bessel filter (100 Hz) and digitized at a 1 kHz sampling

interval per signal using Clampex 9.2 (Axon instruments). Data analyses were performed with Clampfit 9.2 (Axon Instruments).

Selectivity determination by planar bilayer clamp technique. Planar bilayer membranes were formed across an aperture in a delrin barrier by painting lipids (Asolectin, extract from soybeans, 25 mg/mL in *n*-decane) over the 200 μm aperture on the side of a cuvette fitted into a chamber. Cuvette (*trans*) and chamber (*cis*) contained asymmetric buffer solutions (*trans*: 450 mM KCl, 10 mM HEPES, pH = 7; *cis*: 3 M KCl, 10 mM HEPES, pH = 7). Membrane formation was confirmed by the capacitance reading on Bilayer Clamp Amplifier (membranes with a capacitance lower than 100 pF were discarded). An aliquot of a trifluoroethanol solution of the compound (concentration varies depending on the feature of compounds) was stirred into the buffer on the chamber side ("cis" side where the input electrode is immersed) to achieve the desired concentration (0.5 μM). Records were filtered with a 4-pole Bessel filter (100 Hz) and digitized at a 1 kHz sampling interval per signal using Clampex 9.2 (Axon instruments). Data analyses were performed with Clampfit 9.2 (Axon Instruments). Selectivity was calculated by derived Goldman-Hodgin-Katz (GHK) voltage equation (a is ion concentration, R is the universal gas constant, T is the temperature in Kelvin, F is the Faraday's constant):

$$\frac{P(K^+)}{P(Cl^-)} = \frac{a(Cl^-, cis) \exp(-VrF / RT) - a(Cl^-, trans)}{a(K^+, cis) - a(K^+, trans) \exp(-VrF / RT)}$$

when reversal potential $Vr = 14.95$ mV was obtained from *I-V* plot in Figure 3.

Bis-[2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-amine, 2. A solution of *t*-butyldimethylsilyl chloride (5.19 g, 34.4 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise (0 $^\circ\text{C}$) to a solution of diethanolamine (1.65 g, 15.6 mmol), triethylamine (3.80 g, 37.6 mmol) and DMAP (300 mg, 2.40 mmol) in dry CH_2Cl_2 (50 mL). The mixture stirred at rt overnight. Reaction system was washed with water (50 mL) and saturated aq. sodium bicarbonate (50 mL). The organic phase was dried (MgSO_4) and the solvent was evaporated (*in vacuo*) to afford crude product. The crude product was purified by distillation to give the target product as yellow oil (3.50 g, 74%). $^1\text{H-NMR}$: 0.016 (12H, s), 0.85 (18H, s), 2.66-2.70 (4H, m), 3.67-3.71 (4H, m). $^{13}\text{C-NMR}$: -5.13, 18.52, 26.17, 52.02, 62.80.

N,N-Bis-[2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-2-chloro-acetamide, 3. A solution of chloroacetyl chloride (1.50 g, 13.2 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise (0 $^\circ\text{C}$) to a solution of compound 2 (2.00 g, 3.30 mmol) and triethylamine (2.00 g, 19.8 mmol) in dry CH_2Cl_2 (20 mL). The reaction was stirred at rt overnight. The reaction mixture was washed with water (30 mL) and saturated aq. sodium bicarbonate (30 mL). The organic phase was dried (MgSO_4) and the solvent was evaporated (*in vacuo*) to afford crude product as yellow oil.

The crude product was purified by distillation to afford target product as yellow oil (1.92 g, 74%). $^1\text{H-NMR}$: 0.048-0.052 (12H, d, $J = 1$ Hz), 0.88-0.89 (18H, d, $J = 1$ Hz), 3.48 (2H, t, $J = 5$ Hz), 3.62 (2H, t, $J = 5$ Hz), 3.75-3.79 (4H, m), 4.24 (2H, s). $^{13}\text{C-NMR}$: -5.39, -5.30, 18.35, 25.85, 26.00, 26.05, 41.65, 48.97, 51.84, 60.93, 61.49, 197.70.

N,N-Bis-[2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-2-(4'-hydroxy-biphenyl-4-yloxy)-acetamide, 4. 4,4-biphenol (0.564 g, 3.03 mmol) was added into a suspension of compound **3** (1.00 g, 2.52 mmol), sodium carbonate (3.00 g, 28.3 mmol), Potassium iodide(cat.) in butyronitrile (40 mL). The reaction mixture was stirred at reflux temperature for 5 hours. The process was monitored by TLC (hexane: acetone 2:1). The reaction was cooled, filtered and the solvent was evaporated (*in vacuo*). The residue was chromatographed (SiO_2 , 1% MeOH in CH_2Cl_2) to afford the crude product as yellow oil (0.77 g, 55%). The crude product was used directly in the next step without further purification. $^1\text{H-NMR}$: 0.033-0.070(12H, m), 0.88-0.90 (18H, m), 3.545 (2H, m), 3.660 (2H, m), 3.78-3.81 (4H, m), 4.86 (2H, s), 6.82-6.85 (2H, d, $J = 9$ Hz), 6.95-6.98 (2H, d, $J = 9$ Hz), 7.35-7.41 (4H, m).

12-Bromododecanoic acid [3-(12-bromo-dodecanoylamino)-phenyl]-amide, 5. Oxalyl chloride (0.91 g, 7.2 mmol) was added dropwise (0 °C) to a solution of 12-bromododecanoic acid (0.50 g, 1.8 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 2 hours. Toluene (5 mLx2) was added into the residue and evaporated to ensure removal of residue (ClCO)₂. The residue was dissolved in dry CH_2Cl_2 (5 mL) and added dropwise (0°C) to a solution of 1,3-phenylene diamine • 2HCl (0.15 g, 0.83 mmol), Et_3N (2 mL, excess) in dry CH_2Cl_2 (20 mL). The reaction warmed to rt during 20 minutes and stirred overnight. Some target product precipitated directly from the reaction system as white solids. After collecting the precipitates from the system, the solvent was evaporated (*in vacuo*) and the residue was crystallize in CH_2Cl_2 (freeze) to afford more target product. The two portions of target product are combined together (0.44 g, 84%). Melting Point: 120-121 °C. $^1\text{H-NMR}$: 1.25-1.45 (28H, m), 1.72-1.78 (4H, m), 1.83-1.90 (4H, m), 2.37 (4H, t, $J = 7$ Hz), 3.44 (4H, t, $J = 7$ Hz), 7.31-7.44 (3H, m) and 7.86 (1H, s). $^{13}\text{C-NMR}$: 25.80, 27.08, 28.37, 28.95, 29.07, 29.45, 29.55, 29.60, 29.66, 32.86, 33.04, 34.27, 38.06, 45.41, 111.09, 115.47, 129.71, 138.81, 171.83.

6. Compound **5** (0.10 g, 0.16 mmol), compound **4** (0.20 g, 0.36 mmol), sodium carbonate (0.40 g, 3.7 mmol), KI (cat.) in butyronitrile (20 mL) was heated to reflux for 3 days. The reaction system was cooled, filtered and the solvent was evaporated (*in vacuo*) to afford crude product. The crude product was crystallized in MeOH (freeze) to afford target product as slight yellow powder (110 mg, 19%). Melting Point: 78-81 °C. $^1\text{H-NMR}$: 0.028-0.081 (24H, m), 0.87-0.91 (36H, m), 1.29-1.81 (36H, m), 2.28-2.34 (4H, m), 3.53-3.65 (8H, m), 3.78-3.80 (8H,

m), 3.96-4.00 (4H, m), 4.83 (4H, s), 6.93-6.99 (8H, m), 7.26-7.45 (11H, m), 7.79 (1H, s). ¹³CNMR: -5.24, 18.39, 18.49, 25.79, 26.10, 29.47, 29.54, 29.64, 29.69, 38.00, 48.86, 61.28, 61.69, 67.36, 68.28, 114.98, 115.16, 115.40, 127.92, 129.66, 133.39, 134.44, 138.83, 157.50, 158.51, 168.65, 171.82. MS-FAB: m/z calcd: (M+Na) 1609.9912, found: 1609.9950.

1. Concentrated HCl (37.4%, 0.5 mL) was added dropwise to a solution of protected **3** (30 mg, 0.019mmol) in EtOH (5 mL). The solution was stirred at rt for 2 hours. The target product (20 mg, 93%) precipitated from the reaction system as brown solid. Melting Point: >189 °C decomposed. ¹H-NMR (DMSO-d₆): 1.31-1.61 (36 H, m), 2.29-2.32 (4H, m), 3.46-3.63 (16H, m), 4.01 (4H, m), 4.95 (4H, s), 6.97-7.02 (8H, m), 7.20-7.31 (3H, m), 7.51-7.56 (8H, m), 7.96 (1H, s), 9.88 (2H, s). ¹³CNMR: 25.20, 25.27, 36.42, 47.92, 49.49, 58.64, 58.78, 65.66, 67.51, 110.19, 113.97, 114.85, 114.96, 127.03, 127.256, 128.68 132.24, 132.53, 139.60, 157.43, 157.83, 167.71, 171.36.