

Supporting Information for

A polymeric membrane permeabilizer displaying densely packed arrays of crown ether lateral substituents

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Materials. 2-(Hydroxymethyl)-18-crown-6 (95%, Aldrich), malonyl chloride (99%, Fluka), phosphazene base Bu^tP₄ solution (1.0 mol.L⁻¹ solution in hexane, Fluka), thiophenol (≥ 99%, Aldrich), anhydrous dimethyl sulfoxide (99.9%, Aldrich), 1,2-dibromoethane (99%, Aldrich), potassium carbonate (99.9%, Aldrich), sodium bicarbonate (≥ 99.0%, Fluka), dichloromethane (≥ 99.5%, Carlo Erba), dioleoyl-glycero-phosphatidylcholine and dioleoyl-glycero-phosphoethanolamine (Avanti Polar Lipids, Alabaster, AL, USA) were used as received. Tetrahydrofuran (THF) and toluene were dried with sodium-benzophenone and then distilled under argon.

Instrumentation. ¹H- and ¹³C-NMR spectra were recorded in CDCl₃ or CD₃COCD₃ using a Bruker 400 MHz NMR spectrometer. Size Exclusion Chromatography (SEC) experiments were performed in THF containing 0.1 wt % tetra-n-butylammonium bromide (0.8 mL.min⁻¹) at room temperature using a Shimadzu LC-10AD pump, and one PLgel Polymer Laboratories columns (3 μm Mix-E, special column for low molecular weights, separation range: up to 30 x 10³). A Wyatt Technology Optilab Rex interferometric refractometer (690 nm laser. Relative molecular weights were obtained using a polystyrene calibration. Absolute molecular weights were determined by quantitative ¹H-NMR. UV and visible spectra were obtained with a Varian Cary 50Bis instrument.

Synthesis of (1p). Malonyl chloride (1.66 g, 11.0 mmol) was added dropwise to a solution of hydroxymethyl-18-crown-6 (3.00 g, 10.2 mmol) in toluene (8 mL). The mixture was stirred for 3 days at 55 °C. CH₂Cl₂ (100 mL) was added to dissolve the filtrated solution and the resulting organic phase was washed three times with a saturated NaHCO₃ aqueous solution (80 mL) and five times with distilled water (50 mL). The organic phase was then dried over magnesium sulfate and after evaporation, a brownish oil was recovered. The product was extracted with hot pentane (120 mL) in a round bottom flask (about twenty times, until the

extraction yield was close to zero). **1p** (yield = 55.4 %), a pale yellow oil, was recovered after evaporation of the combined pentane extracts

^1H NMR (CDCl_3 , 400 MHz): δ (ppm) = 3.41 (s, 2H, $\text{CH}_2\text{C}(\text{CO}_2\text{R})_2$), 3.50–3.85 (m, 46H, crown ether), 4.15–4.19 (m, 2H, $-\text{CH}_2\text{-crown ether}$), 4.26–4.30 (m, 2H, $-\text{CH}_2\text{-crown-ether}$); ^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) = 40.3 ($-\text{CH}_2\text{C}(\text{CO}_2\text{R})_2$), 63.9 ($-\text{CH}_2\text{-crown-ether}$), 69.41, 69.47, 69.56, 69.64, 69.69, 69.76, 69.79, 69.83, 69.88, 70.03, 76.4 (crown ether), 165.3 ($\text{CH}_2\text{C}(\text{CO}_2\text{R})_2$).

FT-IR (cm^{-1}): 2865, 1732, 1451, 1350, 1252, 1111, 945, 847.

Synthesis of (1). A mixture of (**1p**) (3.6 g, 5.5 mmol), 1,2-dibromoethane (2.05 g, 11.0 mmol), anhydrous carbonate K_2CO_3 (4.55g, 33.0 mmol) and DMSO (4 mL) was stirred vigorously for 3 days at room temperature. 30 mL of water was added to the resulting mixture, and the aqueous phase was extracted with five 30 mL CH_2Cl_2 . The combined CH_2Cl_2 extracts were washed with distilled water (three times), and then dried over magnesium sulfate. CH_2Cl_2 was evaporated and the residue was purified by column chromatography (SiO_2 ; petroleum ether/ EtOAc (1:1, v/v), then EtOAc/MeOH(1:1, v/v)] to give **1** (1.5g, 69.9 %) as a pale yellow oil. Yield = 57.6 %.

^1H NMR (CDCl_3 , 400 MHz): δ (ppm) = 1.48 (s, 4H, $(\text{CH}_2)_2\text{C}(\text{CO}_2\text{R})_2$), 3.62–3.84 (m, 38H, crown ether), 4.13–4.19 (m, 2H, $-\text{CH}_2\text{-crown ether}$), 4.25–4.29 (m, 2H, $-\text{CH}_2\text{-crown ether}$).

^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) = 15.9 ($(\text{CH}_2)_2\text{C}(\text{CO}_2\text{R})_2$), 27.0 ($(\text{CH}_2)_2\text{C}(\text{CO}_2\text{R})_2$), 63.8 ($-\text{CH}_2\text{-crown ether}$), 69.00, 69.39, 69.69, 69.78, 69.85, 69.94, 69.99, 75.98 (crown ether), 168.25 ($\text{CH}_2\text{C}(\text{CO}_2\text{R})_2$).

FT-IR (cm^{-1}): 2865, 1726, 1451, 1299, 1250, 1109, 990, 944, 848, 752.

Polymerization of (1). Filling of the polymerization tube with the reagents prior to its closure was carried out in a glove-box. Monomer (**1**) (1.35 g, 2.05 mmol) was introduced under argon into a polymerization tube fitted with a Rotaflo[®]. THF (1.0 mL), thiophenol (13 μL , 0.13

mmol) and the phosphazene base (260 μL , 1.0 mol.L⁻¹ solution in hexane) were successively added at room temperature. After careful closure of the reaction tube, the mixture was stirred at 60°C and let to react. The reaction was quenched with a 12 mol.L⁻¹ HCl aqueous solution after 24 h. The polymer was purified by dialysis (membrane cut-off of 1000 Da). Poly(**1**) was recovered as a viscous liquid after being dried under vacuum for 24 h.

¹H NMR (CD₃COCD₃, 400 MHz): δ (ppm) = 1.80–1.92 (br, -CH₂CH₂C(CO₂R)₂), 3.56–3.88 (br, crown ether), 4.14–4.44 (br, -CH₂-crown ether).

¹³C NMR (CD₃COCD₃, 100 MHz): δ (ppm) = 28.2 ((CH₂)₂C(CO₂R)₂), 56.4 ((CH₂)₂C(CO₂R)₂), 64.9 (-CH₂-crown ether), 69.4–70.3, 76.3 (crown ether), 170.03 (CH₂C(CO₂R)₂).

FT-IR (cm⁻¹): 2866, 1730, 1452, 1351, 1250, 1185, 1109, 991, 944, 847, 746.

Saturation Experiments. A 0.01 mol/L metal hydroxide solution containing a known amount of picric salt (Li, Na and K) was vigorously shaken in a separatory funnel with an equal volume of a dichloromethane solution of the crown ether (i.e. (**1**), poly(**1**), 7. 10⁻⁵ mol/L). The organic layer was removed and the picrate concentration was measured by UV-spectroscopy, using the extinction coefficients reported by Pederson (λ_{max} = 357 nm, ϵ = 1.5 x 10⁴ in water and λ_{max} = 378 nm, ϵ = 1.8 x 10⁴ in CH₂Cl₂). For each cation-crown system the measurements were carried out at five different picrate concentrations, using picrate:crown ratios of 1:1, 4:1, 7:1, 10:1 and 15:1.

BLM conductance measurements. BLM was formed on a hole (0.15 mm diameter) of a polystyrene chamber (Warner Instruments, Hamden, CT) on the interface of two solutions, *cis* and *trans*. The BLM was prepared from the mixture of lipids dioleoyl-glycero-phosphatidylcholine and dioleoyl-glycero-phosphoethanolamine at the molar ratio of 1:1 in n-decane (20 mg/ml). The *cis/trans* solutions for the measurement of the Na⁺/Cl⁻ BLM permeability ratio were (in mmol/l) 50/250 (or 250/50) NaCl and for the K⁺/Na⁺ permeability

250/250 KCl/NaCl. The solutions were buffered to pH 7.4 by 5/2.5 mmol/l HEPES/Tris. The *cis/trans* solutions for the measurement of the Ca^{2+} and K^{+} BLM permeability were (in mmol/l) 43.6/100 $\text{Ca}(\text{OH})_2/\text{KOH}$. The solutions were buffered to pH 7.4 by 160 mmol/l HEPES. Poly(**1**) was added in the *cis* solution (1-1000 fmol/l), incubated for 20-30 min, then the BLM was broken and formed again and/or the BLM chamber was rinsed with ethanol, dry and BLM was formed. The BLM electrical current was measured by amplifier (Bilayer Clamp, BC-525D, Warner Instrument), filtered at 1 kHz and digitized at 4 kHz by DigiData 1200 (Axon Instruments), stored on PC computer by program FETCHEX (pCLAMP6). The polymer dissolved in DMSO was applied into the *cis* solution. The DMSO volume concentration in the *cis* solution ($\leq 0.1\%$) did not influence BLM current. All the voltages reported here refer to the *trans* side with the *cis* side grounded. To evaluate single channel current, the low pass filter of 250 Hz was used, giving error of single channel amplitude $\sim \pm 0.15$ pA and time resolution ~ 4 ms.

Scheme S1. synthesis of the monomer (1)

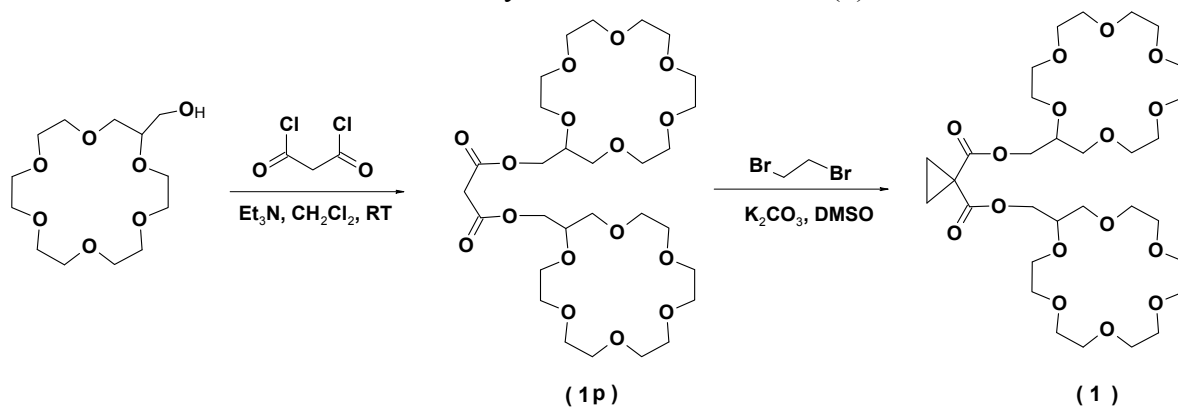
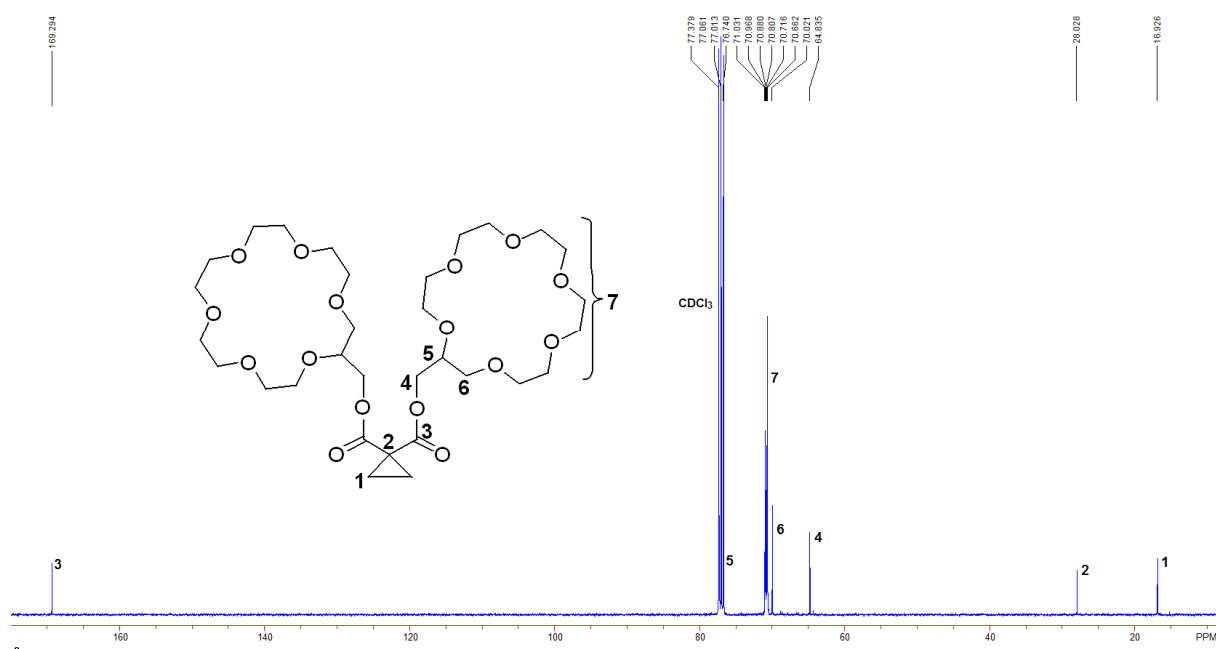
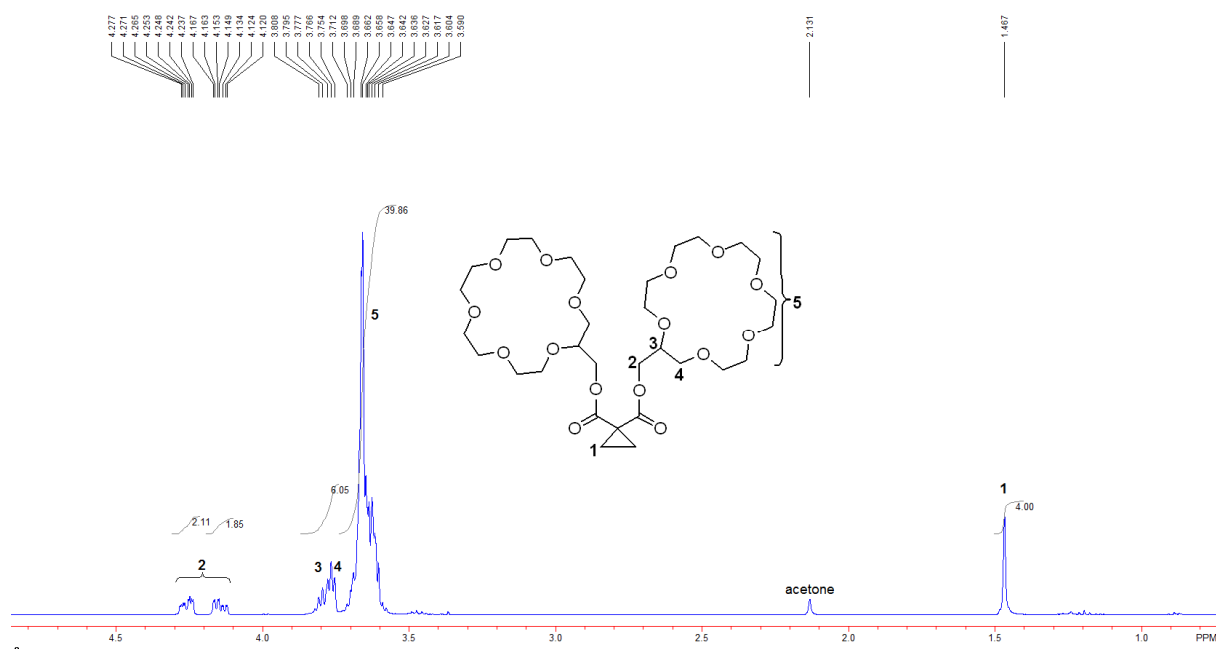


Table S1. Elemental analysis of (1) and poly(1).

(1)	C%	H%	O%
Calculated	54.53	7.97	37.49
Measured	53.56	7.85	38.32
Poly(1)			
Measured	53.56	7.91	36.93



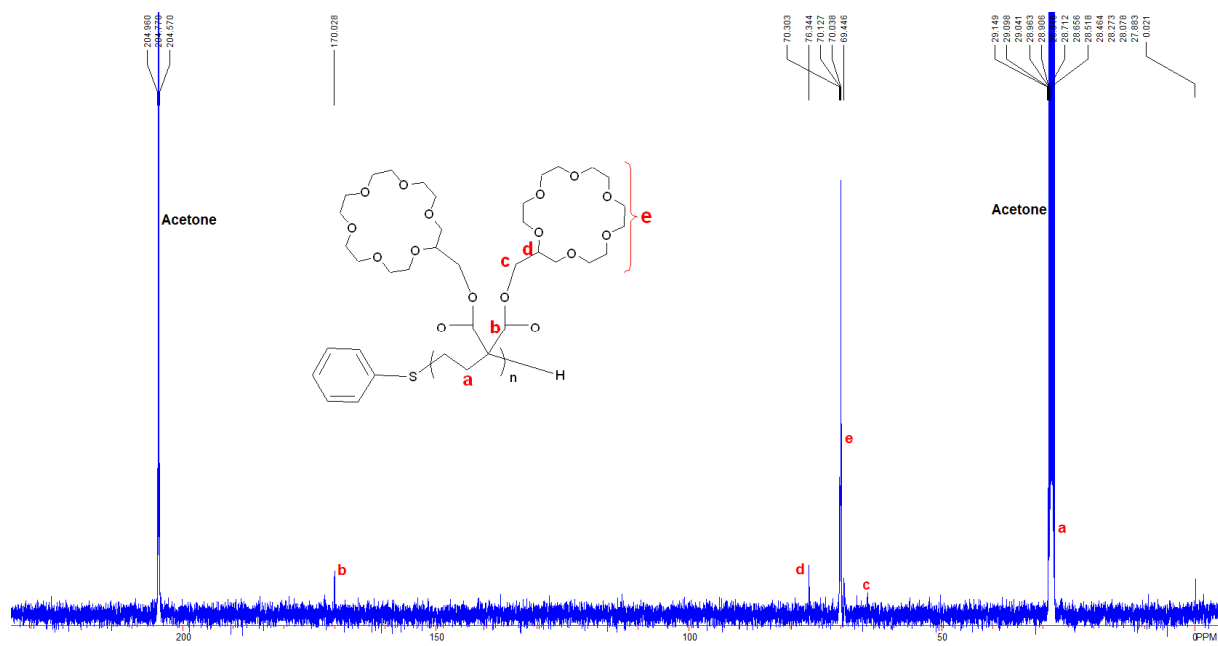


Figure S3. ^{13}C NMR of Poly(1) ((400 MHz, $(\text{CD}_3)_2\text{CO}$, room temperature))

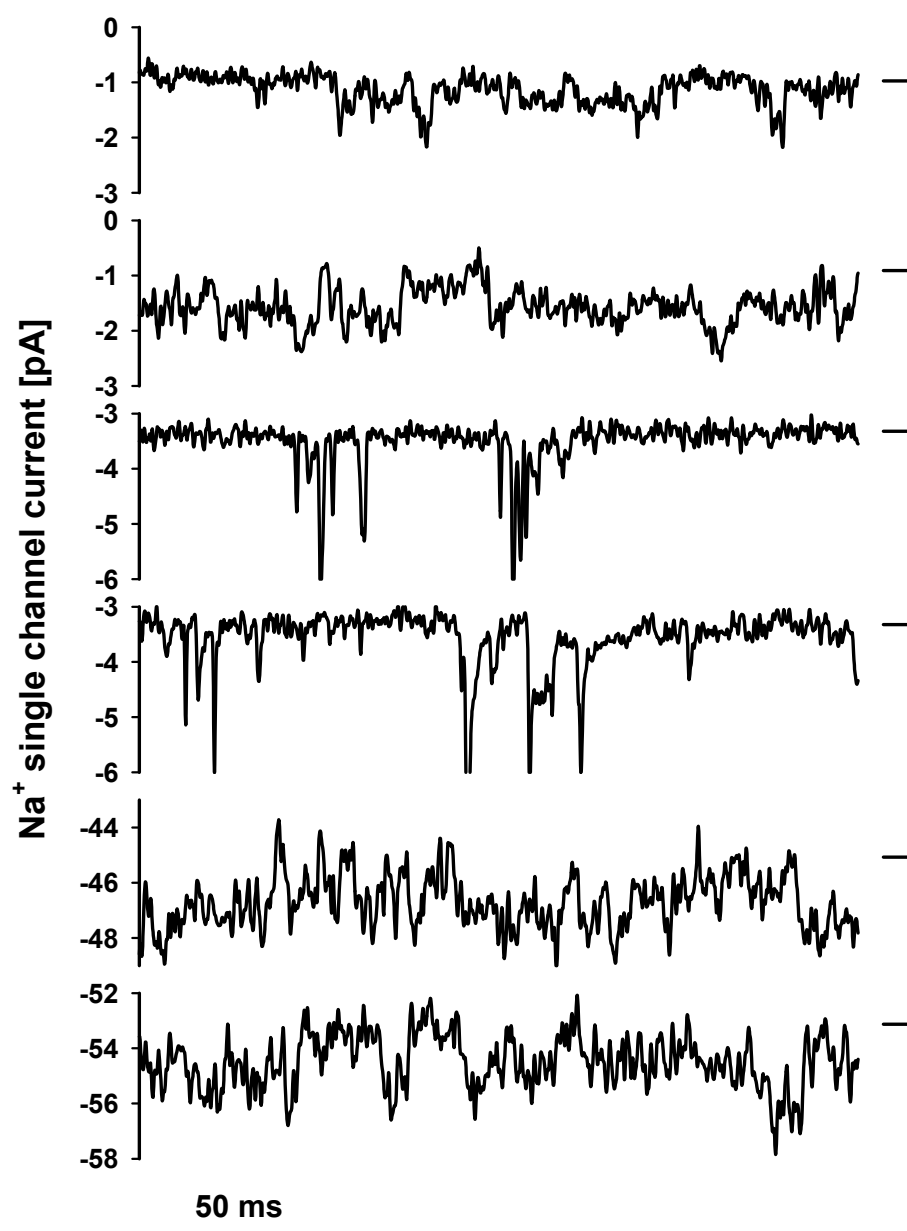


Figure S4. A variation of Na^+ current induced by poly(**1**) incorporated in BLM; 250/50 mmol/l NaCl *cis* / *trans* solutions. Voltage 0 mV. The lines on the right mark the closed state of the channels.

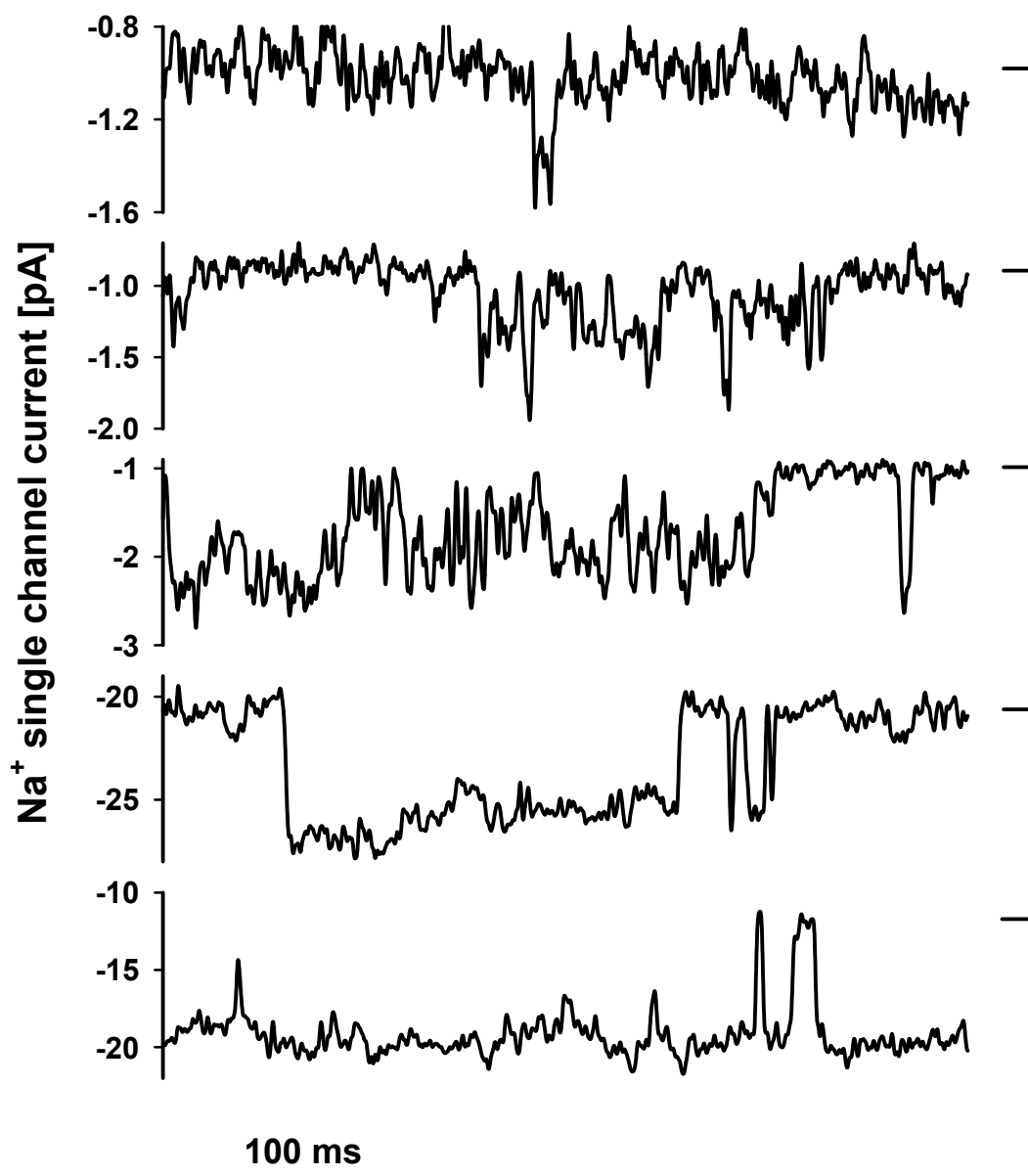


Figure S5. A variation of Na^+ single channel currents induced by poly(**1**) incorporated in BLM; 250/50 mmol/l NaCl *cis* / *trans* solutions. Voltage 0 mV. $E_{\text{rev}} = 30$ mV. Conductance ~ 20 pS (top trace) and ~ 233 pS (bottom trace). The lines on the right mark the closed state of the channels.

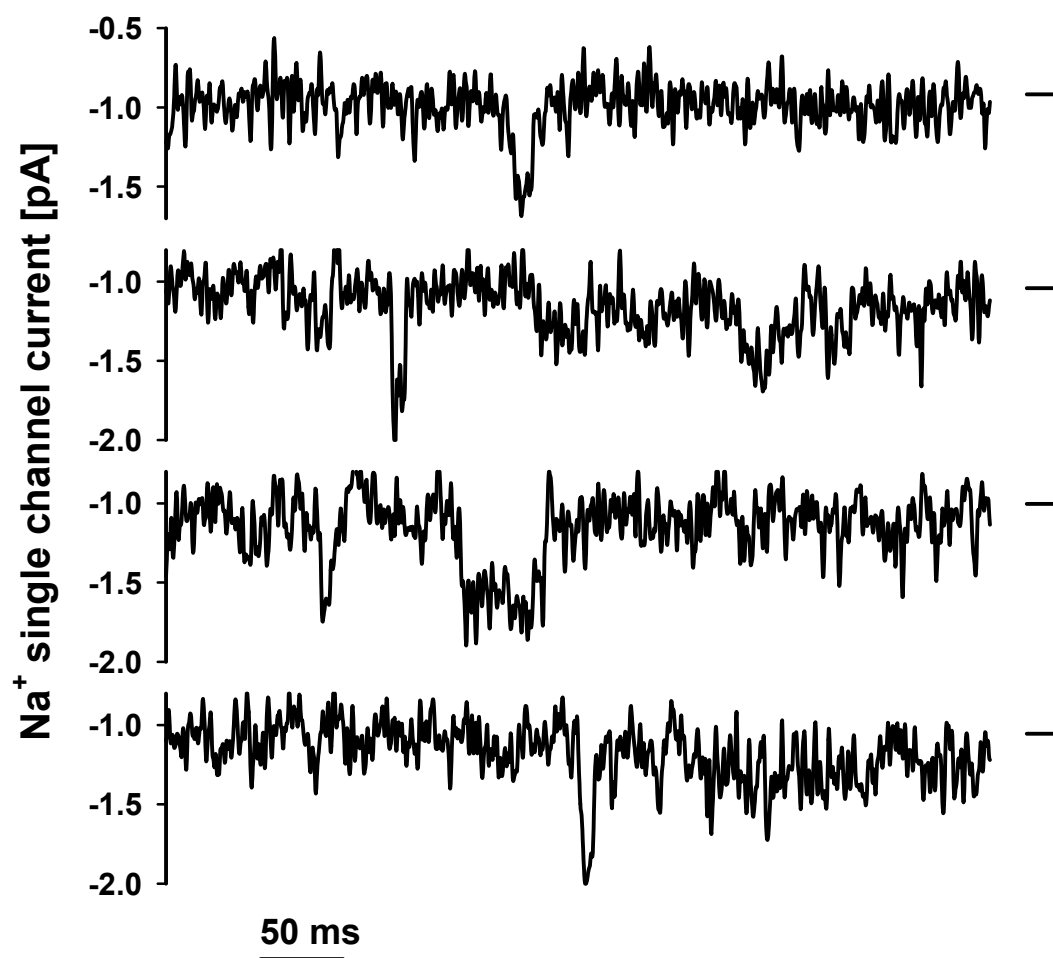


Figure S6. Examples of the lowest Na⁺ single channel currents induced by poly(**1**) incorporated in BLM; 250/50 mmol/l NaCl *cis* / *trans* solutions. Voltage 0 mV. $E_{\text{rev}} = 30$ mV. Conductance ~ 13 pS (top trace) and ~ 53 pS (lower traces). The lines on the right mark the closed state of the channels.

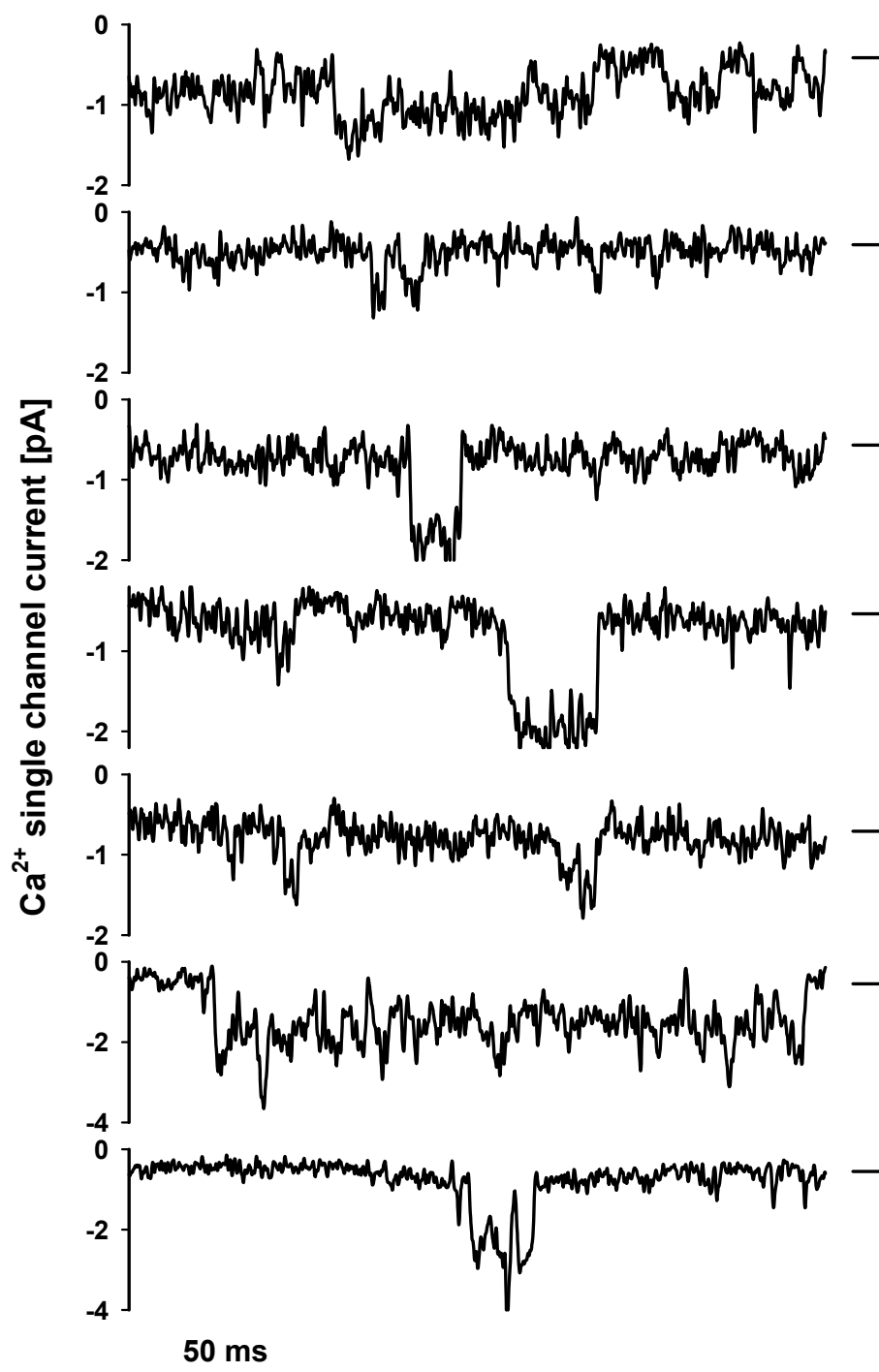


Figure S7. Ca^{2+} single channel current induced by poly(**1**) incorporated in BLM; 43.6/100 mmol/l $\text{Ca}(\text{OH})_2/\text{KOH}$ *cis* / *trans* solutions. Voltage 0 mV. The lines on the right mark the closed state of the channels.

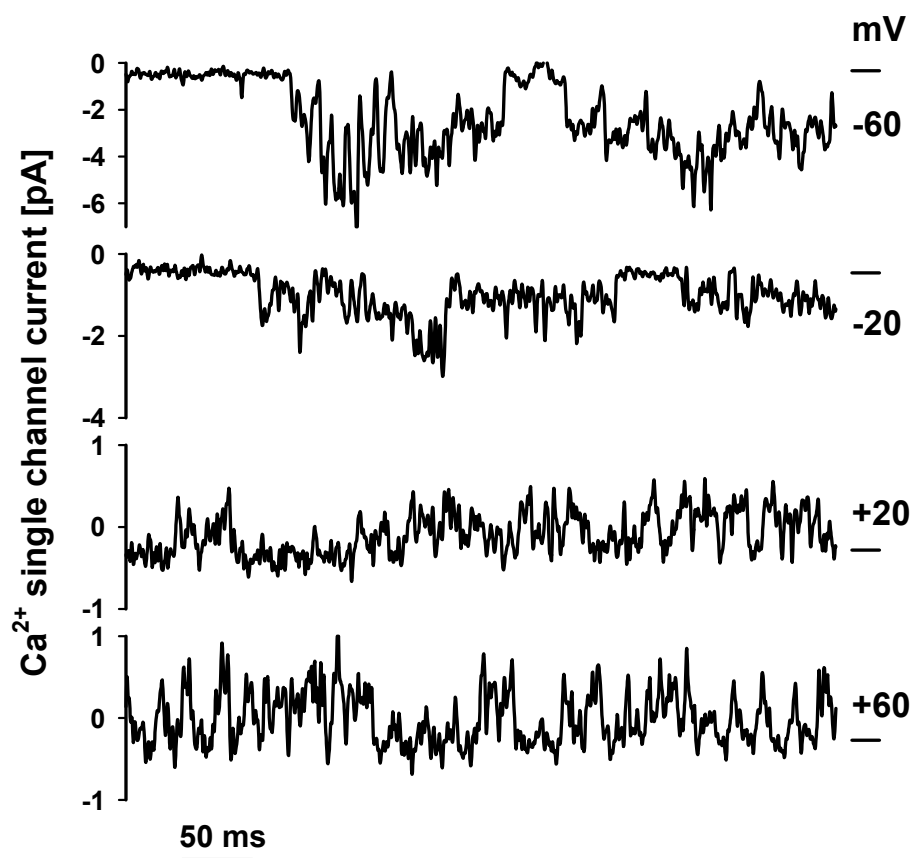


Figure S8. Ca²⁺ and K⁺ single channel currents induced by poly(**1**) incorporated in BLM at the given voltages; 43.6/100 mmol/l Ca(OH)₂/KOH *cis* / *trans* solutions. Ca²⁺ current are observed at -60 and -20 mV. K⁺ current are observed at +20 and +60 mV. The lines on the right mark the closed state of the channels.

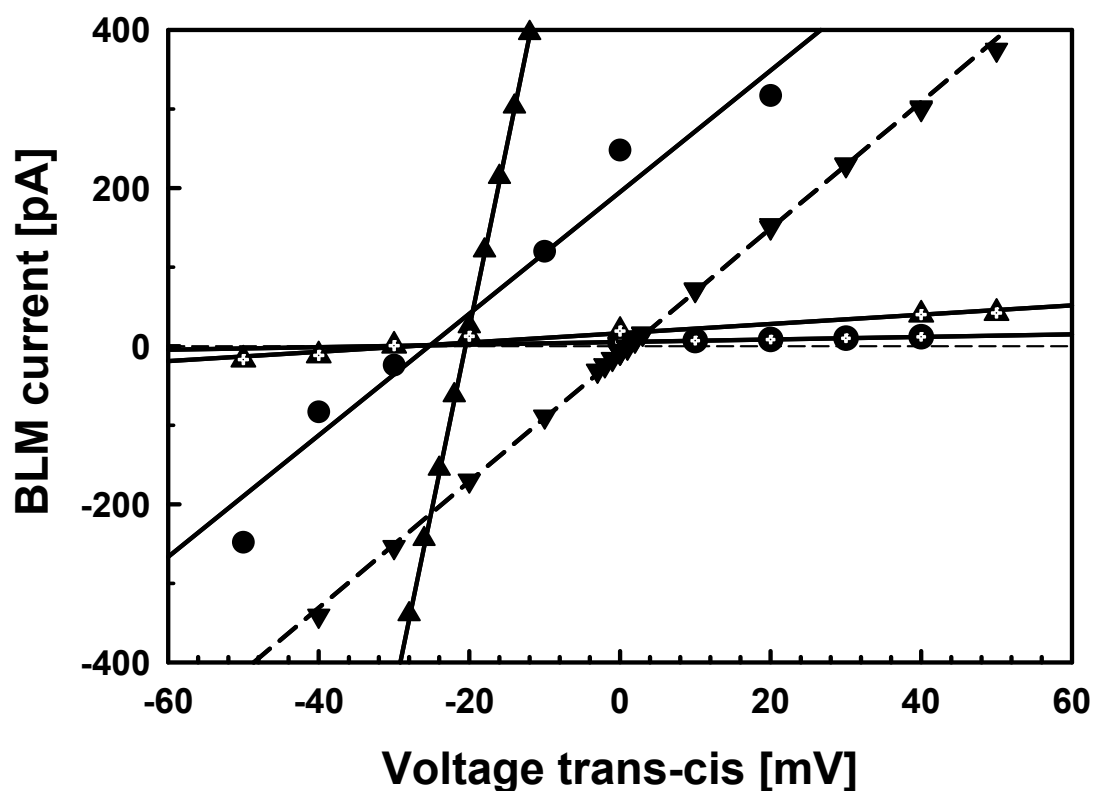


Figure S9. Cation over anion selectivity. Single channel IV profiles; poly(1) 25fmol/l; 50/250 mmol/l NaCl cis / trans solutions. The conductance g , the reversal potential E_{rev} , and the Na^+/Cl^- selectivity ratio P_{Na^+}/P_{Cl^-} were: (○). 161 pS, -32 mV, 11.2/1 . (Δ) 587 pS, -30 mV, 8.6/1. (●). 7.7 nS, -27 mV, 6.3/1 (▲). 47 nS, -20 mV, 3.5/1. K^+/Na^+ selectivity. 250/250 mmol/l KCl/NaCl cis/trans solutions: $g=8.0$ nS, $E_{rev} \sim 1.5$ mV, $P_{Na^+}/P_{K^+} \sim 1$ (▼).