Semithiobambus[6]uril is a Transmembrane Anion Transporter

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Table of Contents

Content	Page
General information	**S1**
Materials	**S1**
Instruments	**S2**
Procedure for anion transport experiments	**S2**
Carboxyfluorescein (CF) assay	**S5**
Fluorescence titration experiments	**S6**
Determination of ion selectivity	**S6**
Cation selectivity assay	**S7**
Electrophysiology measurement	**S8**
References	**S8**

1. General information

Materials

All bambusurils **2**-**5** used in this work were synthesized and fully characterized according to previously reported procedures.[1-3]

Bumbus[6]uril, **2**:[1]
With TBACl: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.41 (12 H), 5.18 (12 H), 3.15 (36 H).

Semithio-bambus[6]uril, **3**:[2]
M.p > 395 ˚C (dec.). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 5.13 (bs, 12 H, CH), 4.87 (bs, 12 H, CH_2), 2.84 (bs, 36 H, CH_3); HRMS (TOF/ESI+), *m/z* calcd for $C_{42}H_{60}N_{24}O_6S_6$: 1188.3452; found: 1188.3470.

Semiaza-bambus[6]uril, **4**:[3]
In the form of a polyiminium n(triflate)/bromide salt: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.22 (s, 6 H, NH), 5.73 (s, 12 H), 5.06 (s, 12 H), 3.30 (s, 36 H), 3.04 ppm (s, 18 H); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 159.6, 158.1, 73.3, 47.7, 34.3, 30.9 ppm; ^{19}F NMR (376 MHz, [D_6]DMSO): δ = –77.9 ppm.
Then, to afford the free base semiaza-bambus[6]uril derivatives we performed neutralization reaction using the polyiminium triflate salt of semiaza-bambus[6]uril (50 mg) and Amberlyst A26 (OH^- form, 50 mg) suspended in methanol (20 mL), and the mixture was stirred at room temperature for 15 min. After filtration the clear

yellowish filtrate was concentrated under reduced pressure to afford an off-white powder identified as free base semiaza-bambus[6]uril derivative, in quantitative yield (>95%). HRMS (TOF/ESI+), m/z calcd for $C_{48}H_{79}N_{30}O_6$ $[M+H]^+$ 1171.6799; found: 1171.6770.

Semithio-bambus[4]uril, **5**:[2]
M.p. 444 °C (dec). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 5.91 (s, 8 H, CH), 4.96 (s, 8 H, CH_2), 3.22 (s, 24 H, CH_3); ^{13}C NMR (100 MHz, [D_6]DMSO) δ = 181.7 (4 C), 159.7 (4 C), 74.2 (8 C), 50.9 (4 C), 33.1 (8 C); HRMS (TOF/ESI$^+$), *m/z* calcd for $C_{28}H_{40}N_{16}O_4S_4$: 792.2301; found: 792.2305.

Instruments

Fluorescence spectra were recorded with a Shimadzu RF-5301PC fluormeter. A planar lipid bilayer (BLM) Workstation (Warner Instruments) integrated with bilayer clamp amplifier (BC-535) was used for electrophysiology measurements. Currents were measured and collected by ML846 data acquisition system (low pass filter = 1 kHz) with 8 pole Bessel filter.

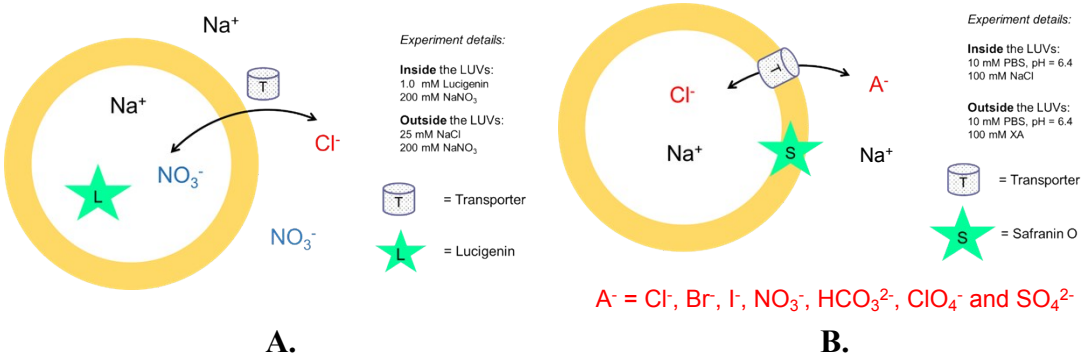

Figure S1. Schematic representations of: **A.** chloride transport (lucigenin assay) and **B**. anion selectivity (safranine O assay).

2. Procedure for anion transport experiments

Lucigenin entrapped LUVs were prepared as follows: 10 mg POPC (or POPC with cholesterol) was first dissolved in $CHCl_3$ (3 mL) and dried under a stream of N_2 to form a thin lipid film. The resultant thin film was then dried under high vacuum for 3 hours. The lipid was hydrated with aqueous $NaNO_3$ (200 mM) solution that contained lucigenin (1 mM) at 40 ˚C for 2 h followed by 10 freeze-thaw cycles with liquid nitrogen and thermostat-regulated water bath. The resulting suspension was then extruded though a polycarbonate membrane (0.22 μm) before purification on Sephadex G-50 to remove the excess vesicular dye. The same lipid hydration solution lacking the fluorescence probe was used as the eluent. The obtained vesicles were stored below 4 ˚C and used within 24 hours. The lipid concentration in each sample was 0.3 mM.

These vesicles were then added to a solution containing NaCl (25 mM) and $NaNO_3$ (200 mM) to generate a higher chloride concentration outside the vesicles. To

examine the transmembrane chloride transport activity of **2-5**, the mixture of lucigenin containing vesicles (50 μL) was futher diluted by adding it into the fluorimetric cell with aqueous $NaNO_3$ (200 mM, 1.95 ml) containing NaCl (25 mM). The fluorescence emission was continuously monitored at 503 nm (excited at 372 nm) and then 10 μL acetonitrile solution of the transporter was added with gentle stirring. Finally, a Triton X-100 water solution (10 μl, 50%) was added to achieve complete lysis of the vesicles. For Cl^- transport experiments with SO_4^{2-} as background anion, Na_2SO_4 (100 mM) was used instead of $NaNO_3$ (200 mM).

By using E_0 and E_∞ to represent the initial and final emission intensity, the collected data of the fluorescence time course was normalized in accordance to the equation:

$$R_f = (E_0 - E_t)/(E_0 - E_\infty).$$

The relative fluorescence R_f at t = 300 s was then analyzed with Hill equation to produce the Hill coefficient *n* and effective concentration EC_{50}.[4] In the Hill equation, y is the R_f at 5 min and x is the transporter concentration. Vmax is the maximum chloride flux possible (usually 100%), n is the Hill coefficient and k is the transporter concentration reach 50% of Vmax at 5 mins. These parameters, Vmax, k and n, were fitted by a non-linear regression model.

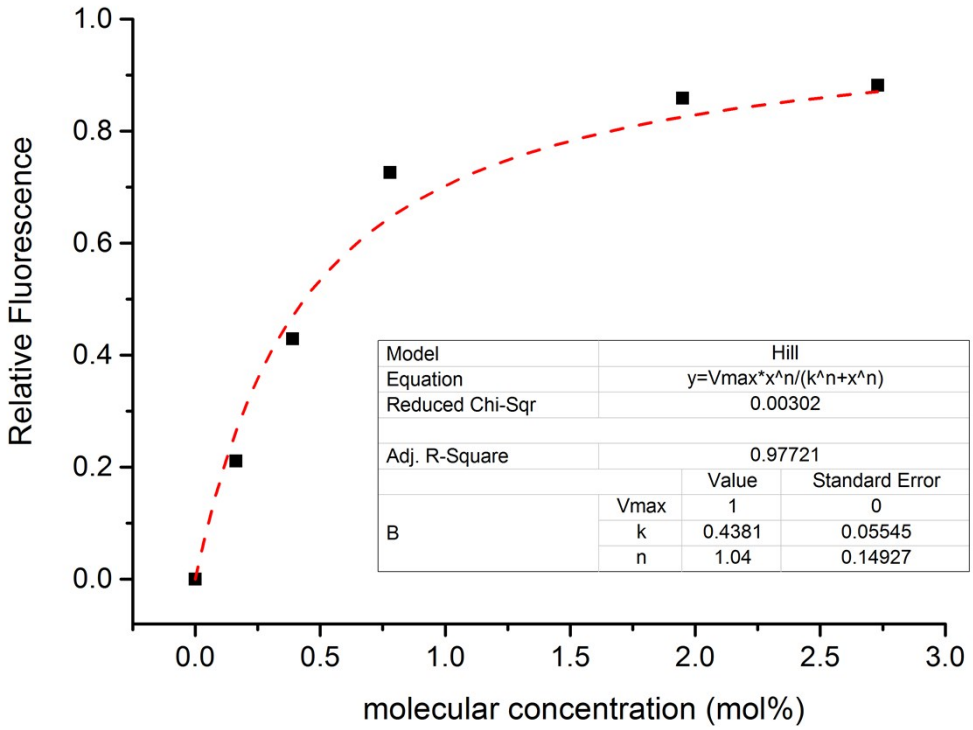

Figure S2. Hill plot analysis of chloride transport promoted by varying concentrations of **3**.

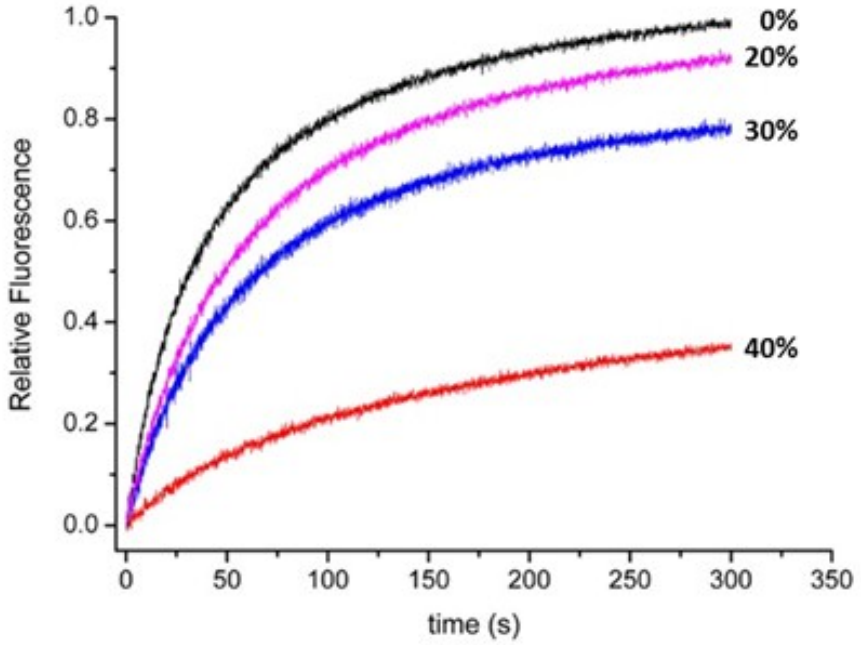

Figure S3. Representative traces of normalized chloride transport (POPC-lucigenin assay) under fixed concentration of **3** (4.0 mol%) and variable cholesterol content.

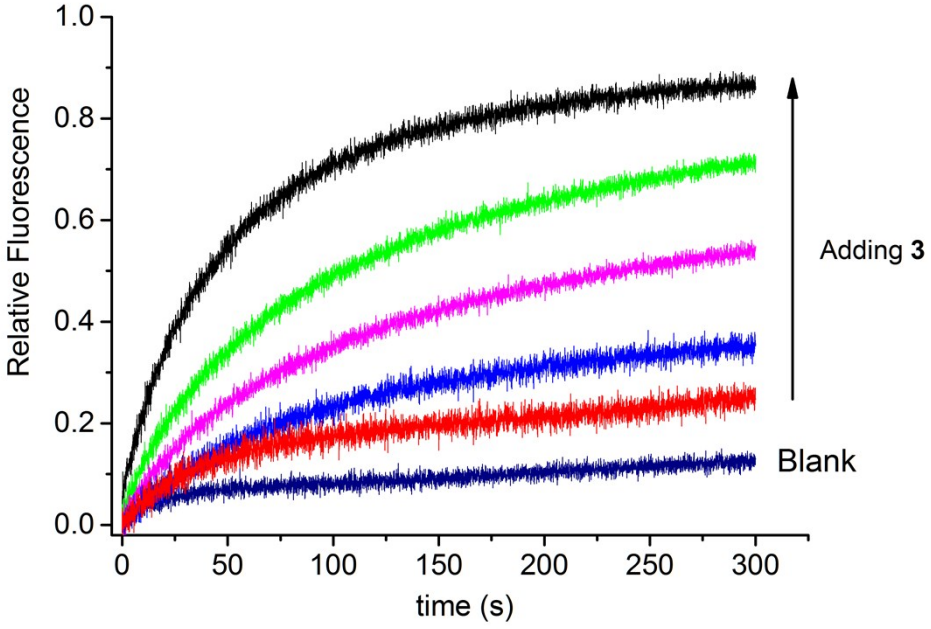

Figure S4. Representative traces of normalized chloride transport (POPC-lucigenin assay) with various concentrations of **3** (mol%) using SO_4^{2-} as the background anion. POPC vesicles loaded with 100 mM Na_2SO_4 and 1 mM lucigenin were suspended in a solution containing 100 mM Na_2SO_4 with 25 mM NaCl. The concentrations of **3** were 0.1, 0.3, 1.2, 2.5 and 4.0 mol% respectively.

3. Carboxyfluorescein (CF) assay[4]

POPC (10 mg) was first dissolved in $CHCl_3$ (3 mL) and dried under a stream of N_2 to form a thin lipid film. The resultant thin film was then dried under high vacuum for 3 hours. The lipid was hydrated with aqueous NaCl (10 mM, 1 mL) buffered at pH 7.5 with PBS (10 mM) and 5(6)-carboxyfluorescein (50 mM) at 40 ˚C for 2 h followed by 10 freeze-thaw cycles using liquid nitrogen and thermostat-regulated water bath. The resulting suspension was then extruded though a polycarbonate membrane (0.22 μm) before purification by Sephadex G-50 to remove the excess vesicular dye using aqueous NaCl (10 mM) buffered at pH 7.5 with PBS (10 mM) as eluent. The obtained vesicles were stored below 4 ˚C and used within 24 hours. The lipid concentration in each sample was 0.3 mM.

These vesicles mixture (50 μL) were then diluted by addition to a fluorimetric cell containing solution (1.95 mL) of NaCl (100 mM) buffered at pH 7.5 with PBS (10 mM). The fluorescence emission was continuously monitored at 517 nm (excited at 492 nm) and then 10 μL DMSO solution of the transporter was added with gentle stirring. After 300 s, a Triton X-100 water solution (10 μl, 50%) was added to achieve complete lysis of the vesicles.

By using E_0 and E_∞ to represent the initial and final emission intensity, the collected data of the fluorescence time course was normalized in accordance to the equation:

$$R_f = (E_t - E_0)/(E_\infty - E_0).$$

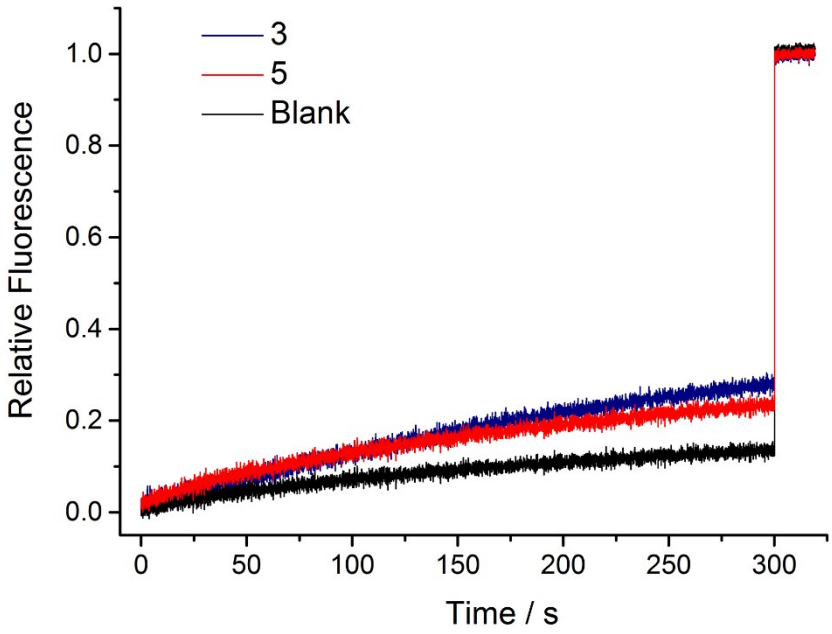

Figure S5. Representative traces of CF leakage caused by **3** and **5** (2.7 mol%). DMSO was used as blank.

4. Fluorescence titration experiments

Fluorescence titration experiments of safranin O against various concentrations of semithiobambus[6]uril were performed. A mixture of safranin O (150 nM) containing POPC lipid vesicles (10 μL) and 100 mM NaCl inside and outside the vesicle (lipid concentration of 1.2 mM) were mixed with incremental amounts of semithiobambus[6]uril.

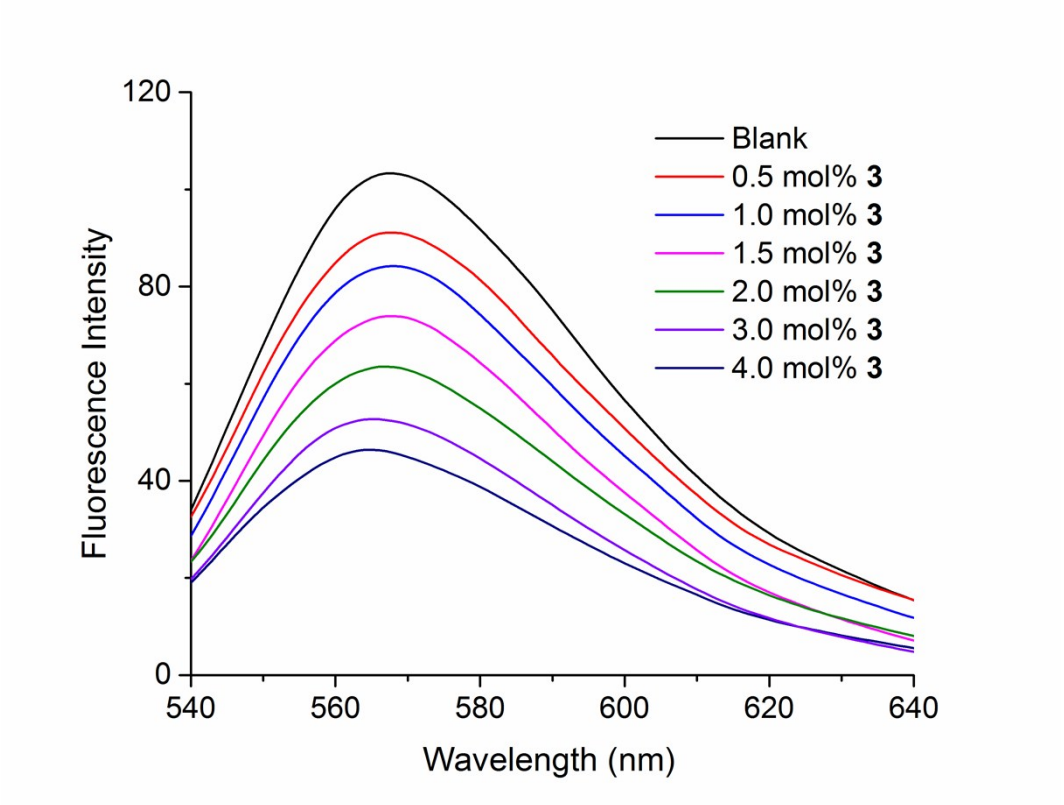

Figure S6. Fluorescence titration of safranin O with increased amount of semithiobambus[6]uril in the presence of POPC-LUVs.

5. Determination of ion selectivity [5]

For the determination of anion selectivity, the following safranin O assay protocol was used. POPC (10 mg) was first dissolved in $CHCl_3$ (3 mL) and dried under a stream of N_2 to form a thin lipid film. The resultant thin film was then dried under high vacuum for 3 hours. The lipid was hydrated with aqueous NaCl (100 mM, 1 mL) buffered at pH 6.4 with PBS (10 mM) at 40 ˚C for 2 h followed by 10 freeze-thaw cycles using liquid nitrogen and thermostat-regulated water bath. The resulting suspension was then extruded though a polycarbonate membrane (0.22 μm). The obtained vesicles were stored below 4 ˚C. The lipid concentration in each sample was 1.2 mM.

Then the vesicles mixture (10 μL) was further diluted by adding it into the fluorimetric cell with aqueous (1.99 mL) NaA (100 mM, A = Cl^-, Br^-, I^-, NO_3^-,

HCO_3^{2-}, ClO_4^- and SO_4^{2-}) buffered at pH 6.4 with PBS (10 mM) and safranine O (150 nM). The fluorescence emission was continuously monitored at 581 nm (excited at 522 nm) and then a DMSO solution of **3** (0.75 mol%, 10 μL) was added with gentle stirring. Finally, a Triton X-100 water solution (10 μl, 50%) was added to achieve complete lysis of the vesicles.

By using E_0 and E_∞ to represent the initial and final emission intensity, the collected data of the the fluorescence time course was normalized in accordance to the equation: $R_f = (E_0 - E_t)/(E_0 - E_\infty)$ or $R_f = (E_t - E_0)/(E_\infty - E_0)$.

6. Cation selectivity assay

The above described POPC-LUVs ⊃ (section 4, 10 μL) was added into a clean and dry fluorescence cuvette containing mixture (1.99 mL) of XCl (100 mM where X = Li^+, Na^+, K^+, Rb^+ or Cs^+) buffered at pH 6.4 with PBS (10 mM) and safranine O (150 nM). The fluorescence emission was continuously monitored at 581 nm (excited at 522 nm) and then a DMSO solution of **3** (0.75 mol%, 10 μL) was added with gentle stirring. Finally, a Triton X-100 water solution (10 μl, 50%) was added to achieve complete lysis of the vesicles. By using E_0 and E_∞ to represent the initial and final emission intensity, the collected data of the fluorescence time course was normalized in accordance to equation: $R_f = (E_0 - E_t)/(E_0 - E_\infty)$ or $R_f = (E_t - E_0)/(E_\infty - E_0)$.

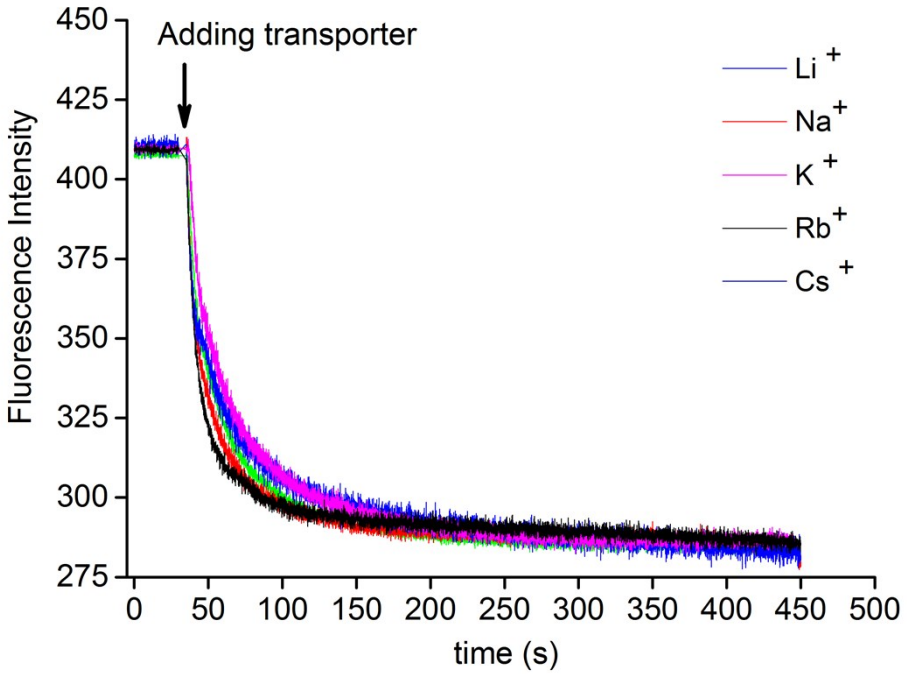

Figure S7. Representative traces of normalized chloride transport activity of **3** using the potential-sensitive dye safranine O (ex 480 nm, em 520 nm) with various cations.

DMSO solutions of **3** (10 μl, 2.7 mol%) were added at t = 30 s. POPC vesicles loaded with NaCl (100 mM) were suspended in a solution containing safranine O (150 nM) and MCl (100 mM), M = Li, Na, K, Rb, Cs.

7. Electrophysiology measurement.[6]

A chloroform solution of EYPC-LUVs was dried under a stream of N_2 and then re-suspended in decane (25 mg/ml). The planer phospholipid bilayer membrane formed by spreading the decane solution on the 200-μm aperture, which links two fluid filled chambers, a polystyrene cup and a chamber. The cup (cis, ground) and chamber (trans) are filled with saline solution. Ag/AgCl electrodes were used to apply voltages and record membrane currents. The examined channel in DMSO was added to the cis cup and the solution was stirred for 5 min.

References

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