Octafluorocalix[4]pyrrole: a chloride/bicarbonate and chloride/nitrate anion antiporter

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Experimental Procedures

Preparation of POPC Vesicles

A lipid film of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Genzyme) was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with a metal chloride (MCl) salt solution (489 mM MCl and 5 mM phosphate buffer, pH 7.2). The lipid suspension was then subjected to seven freeze–thaw cycles and allowed to age for 30 min at room temperature before extruding 25 times through a 200 nm polycarbonate membrane. The resulting unilamellar vesicles were dialyzed against the external medium to remove unencapsulated MCl salts.

Chloride Transport Assays

Unilamellar POPC vesicles, prepared as described above, were suspended in 489 mM $NaNO_3$ or 162 mM Na_2SO_4 solution buffered to pH 7.2 with 5 mM sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment and the chloride efflux was monitored using a chloride selective electrode (Accumet). At 5 min, the vesicles were lysed with 50 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 7 min.

Bicarbonate Assay

Unilamellar POPC vesicles filled with 489 mM NaCl or CsCl solution buffered to pH 7.2 with 20 mM sodium phosphate salts, prepared as described above, were suspended in 162 mM Na_2SO_4 solution buffered to pH 7.2 with 20 mM sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment and the chloride efflux was monitored using a chloride selective electrode (Accumet). At 2 min, $NaHCO_3$ (1.2 M in Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts) was added so that the outer solution had 40 mM $NaHCO_3$. At 8 min, the vesicles were lysed with 50 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 10 min.

Mobility Assay

Preparation of DPPC vesicles

A lipid film of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Genzyme) was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with NaCl solution (489 mM, containing 5 mM phosphate buffer, pH 7.2) and warmed to 45 °C. The lipid suspension was then subjected to seven freeze-thaw cycles, in which the suspension was warmed to 45 °C after each freezing. The vesicles were allowed to age at 45 °C for 30 min before extruding 25 times at 45 °C through a 200 nm nucleopore polycarbonate membrane. The resulting unilamellar vesicles were dialyzed at room temperature against 162 mM Na_2SO_4 solution buffered to pH 7.2 with 5 mM sodium phosphate salts to remove unencapsulated NaCl.

Chloride Transport Assay in DPPC Vesicles

Unilamellar DPPC vesicles, prepared as described above, were suspended in 162 mM Na_2SO_4 solution buffered to pH 7.2 with 5 mM sodium phosphate salts. The lipid concentration was 1 mM.

Assay at 45 ℃

The lipid sample prepared as described above was brought to 45 °C using a water bath. A DMSO solution of the carrier molecule (10 mM, 4% by mass of lipid) was added to start the experiment and chloride efflux was monitored using a chloride sensitive electrode (Accumet). At 2 min, a pulse of $NaNO_3$ solution buffered to pH7.2 with sodium phosphate salts was added such that the final concentration of $NaNO_3$ in the lipid solution was 40 mM. At 7 min, the vesicles were lysed with 50 μl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 v/v water:DMSO) and a total chloride reading was taken at 9 min.

Assay at 37 ℃

The experiment was initially started at 45 °C and monitored as described above. At 2 min, prior to the addition of $NaNO_3$, the system was cooled to 37 °C. A pulse of $NaNO_3$ solution buffered to pH 7.2 with sodium phosphate salts was then added such that the final concentration of $NaNO_3$ in the lipid solution was 40 mM. After a further 5 min, the vesicles were lysed with 50 μl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 v/v water:DMSO) and a total chloride reading was taken after a further 2 min.

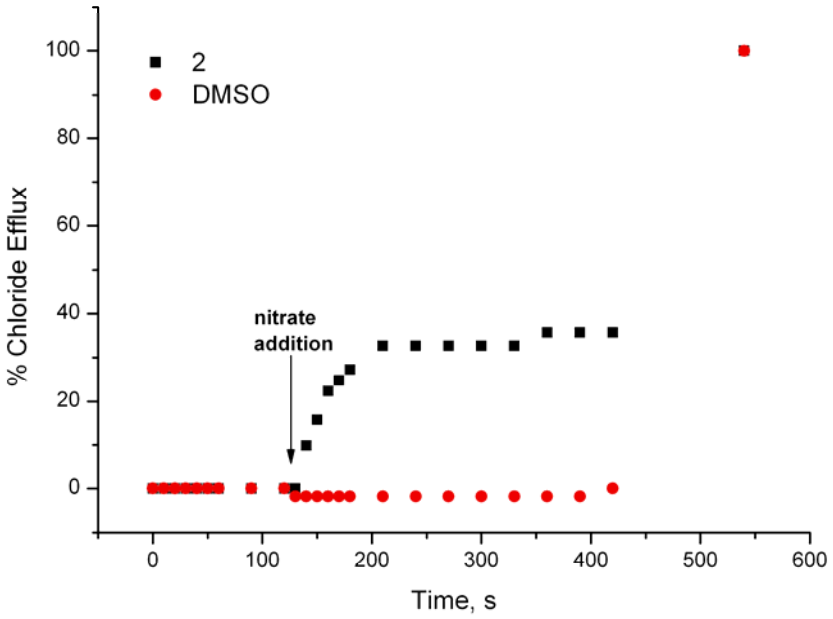

Figure S1 DPPC Mobility assay at 45˚C (see above for experimental conditions).

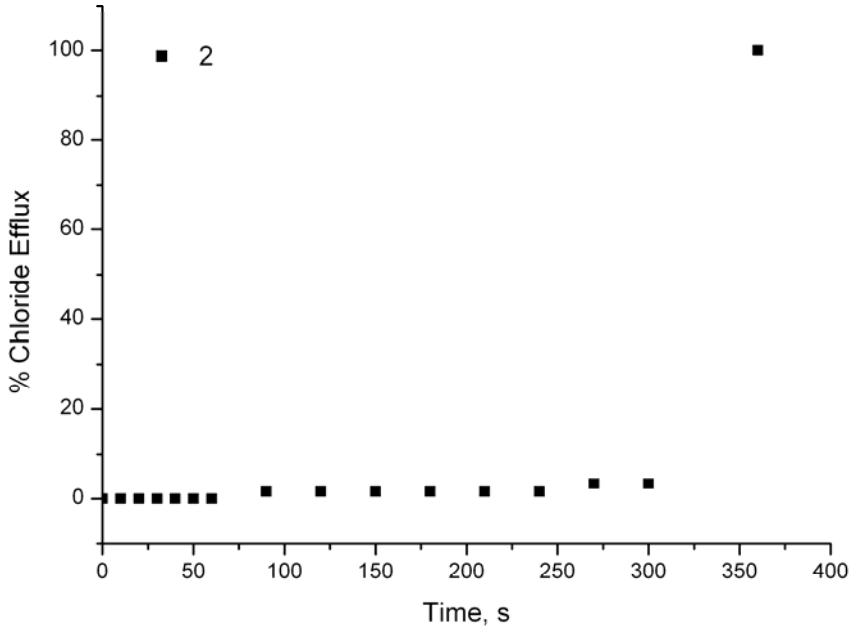

Figure S2 DPPC Mobility assay at 37˚C (see above for experimental conditions).

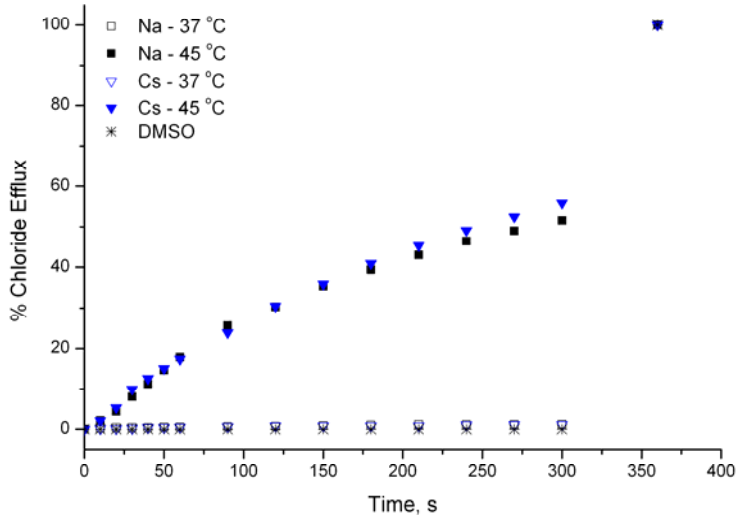

Figure S3 Chloride efflux promoted by **2**, at 0.04 molar equivalents of carrier to lipid, across unilamellar vesicles made of DPPC loaded with 489 mM NaCl (■/□) or CsCl (▼/▽) buffered to pH 7.2 with 5mM phosphate at 37°C (open symbols) and 45°C (closed symbols). The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials. After 300 s, the vesicles were lysed with 50 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 360 s.

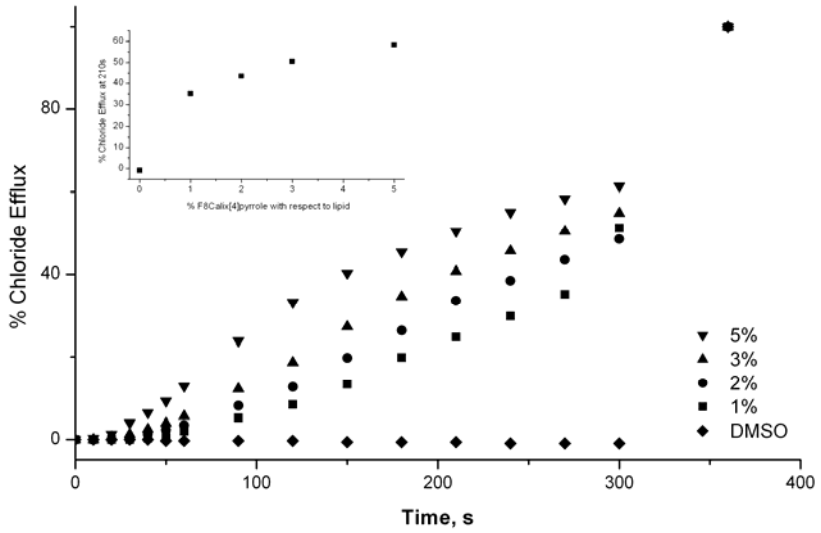

Figure S4 Chloride efflux promoted by 0.01 (■), 0.02 (●), 0.03 (▲) and 0.05 (▼) molar equivalents of **2** across unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials. The large frame shows the chloride efflux as a function of time at different carrier concentrations, while the inset shows the chloride efflux recorded at 210 s as a function of carrier concentration plotted as mole percent with respect to the lipid. After 300 s, the vesicles were lysed with 50 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 360 s.

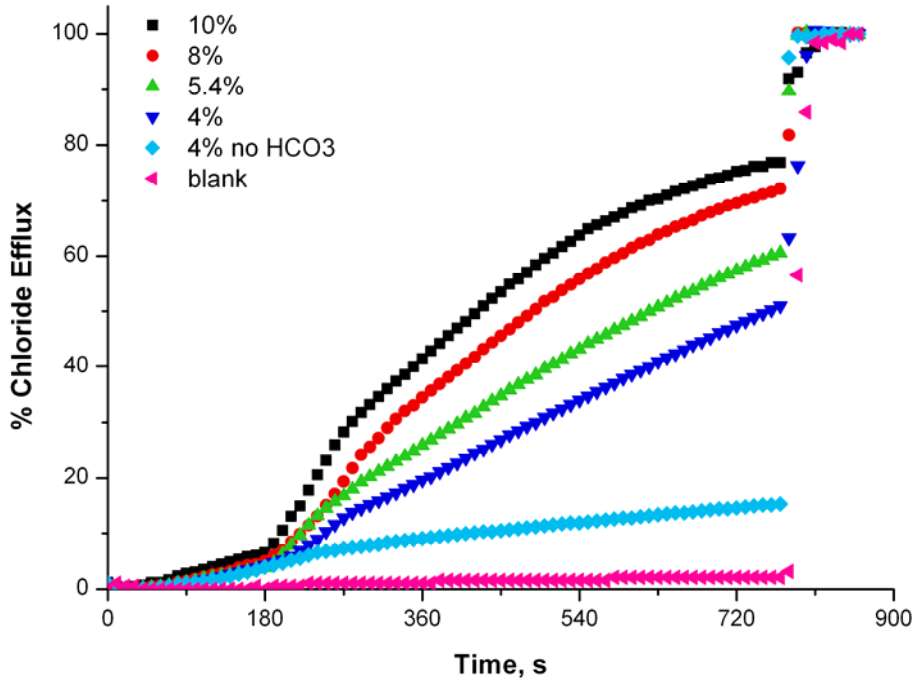

Figure S5 Chloride efflux promoted by 0 (◀), 0.04 (▼), 0.054 (▲), 0.08 (●) and 0.10 (■) molar equivalents of **2** across unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 20mM phosphate upon addition of a $NaHCO_3$ pulse, to make the extravesicular bicarbonate concentration 40 mM. Chloride efflux promoted by 0.04 molar equivalents of **2** (◆) without the addition of bicarbonate. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM phosphate. Each point represents the average of three trials. After 780 s, the vesicles were lysed with 50 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 800 s.

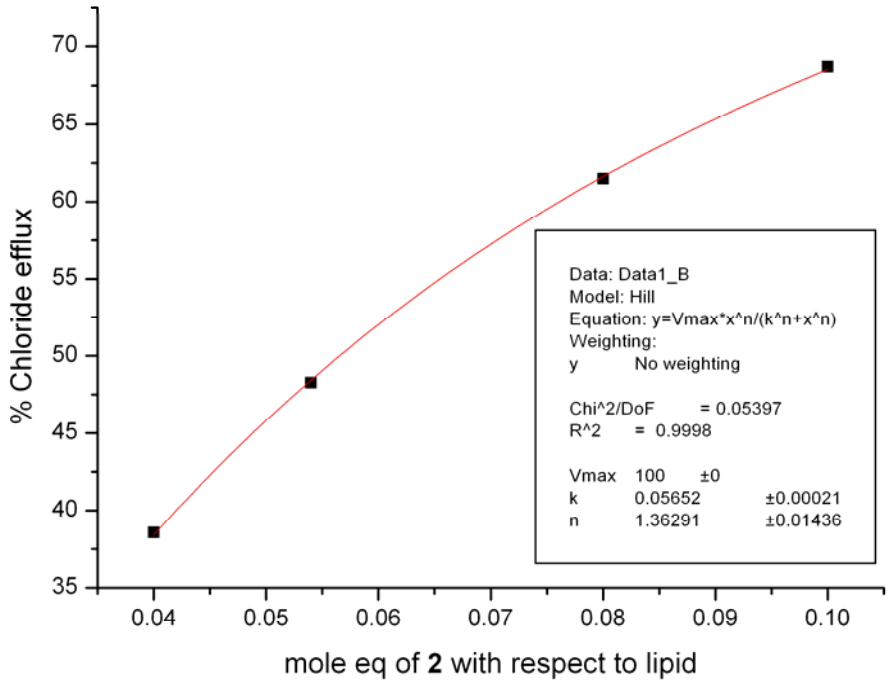

Figure S6 Chloride efflux observed at 600s after addition of **2** at 0.04, 0.054, 0.08 and 0.10 molar equivalents to unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 20mM phosphate. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM phosphate and 40mM $NaHCO_3$. Each point represents the average of three trials. The data is fitted using the Hill equation with V_{max} = 100 (line). The Hill coefficient is 1.36 and the molar equivalents of **2** with respect to lipid ratio at which 50% efflux is observed (EC_{50}) is 0.057.

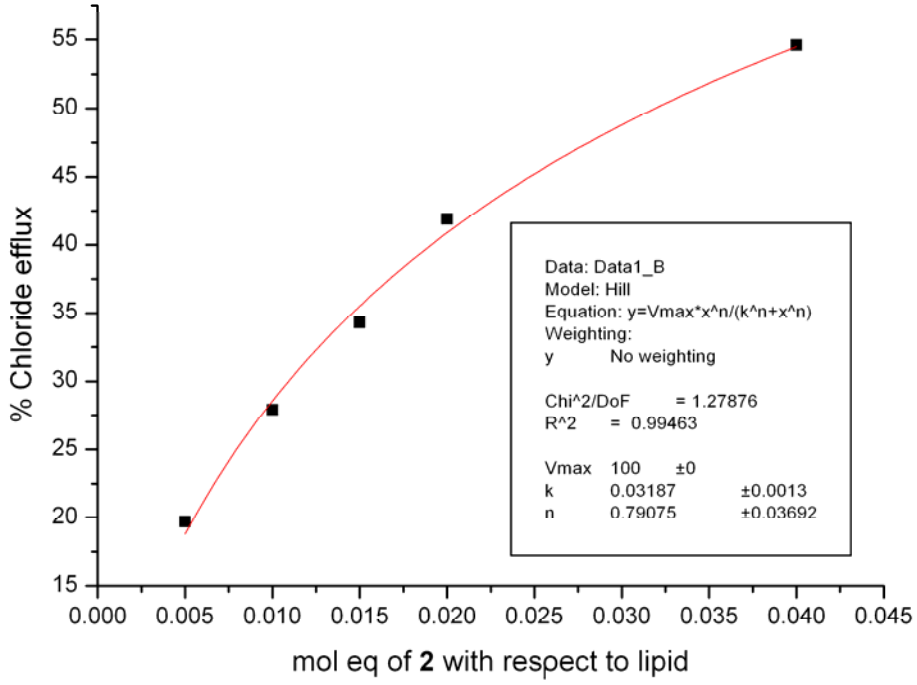

Figure S7 Chloride efflux observed at 270s after addition of **2** at 0.005, 0.01, 0.015, 0.02 and 0.04 molar equivalents to unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials. The data is fitted using the Hill equation with V_{max} = 100 (line). The Hill coefficient is 0.79 and the molar equivalents of **2** with respect to lipid ratio at which 50% efflux is observed (EC_{50}) is 0.031.

U-tube experiments

U-tube experiments were performed using a chloride selective electrode to monitor any transport from Aq. 1 (Donor) to Aq. 2 (Acceptor). The electrode, which was calibrated before and after use, provides readout as mV, with lower numbers corresponding to greater chloride anion concentrations.

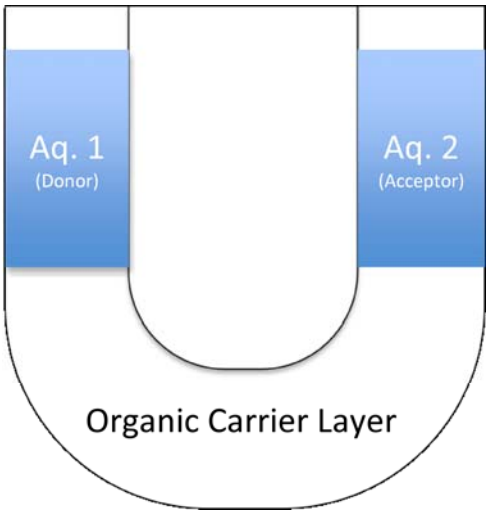

Figure S5 Single U-tube experiment used in these studies.

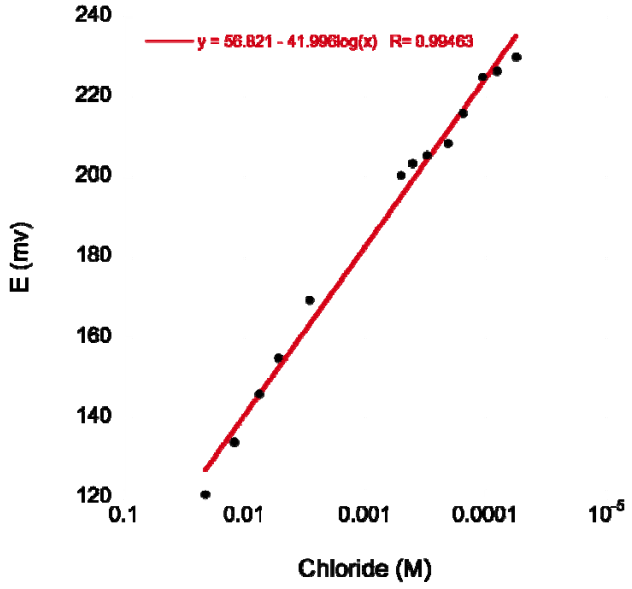

Figure S6 A representative calibration graph for the chloride anion-selective electrode used for the U-tube transport studies.

Experiment 1:

Aqueous 1: 5 mM phosphate buffer, pH: 7.2 and 0.5 M NaCl (5 mL)

Aqueous 2: 5 mM phosphate buffer, pH: 7.2 and 0.5 M $NaNO_3$ (5 mL)

Organic Carrier Layer: 1 mM octafluorocalix[4]pyrrole **2** in CH_2Cl_2 (10 mL)

Table S1 Experiment 1

Time	E (mV)
Initial	~235
5 h	247-249
15 h	243-244
43 h	230-232
65 h	194
89 h	199-200
118 h	185-190
142 h	193-194
195 h	195

Experiment 2:

Aqueous 1: 20 mM phosphate buffer, pH: 7.2 and 0.5 M NaCl (10 mL)

Aqueous 2: 20 mM phosphate buffer, pH: 7.2 and 0.5 M Na_2SO_4 (10 mL): After 24 hours, $NaHCO_3$ was added (bringing the concentration to 140 mM $NaHCO_3$).

Organic Carrier Layer: 1 mM octafluorocalix[4]pyrrole **2** in CH_2Cl_2 (30 mL)

Table S2 Experiment 2

Time	E (mV)
Initial	253
4 h	259-260
18 h	260
22 h	245-246
26 h	231-233
30 h	231-233
44 h	214-215
50 h	209-210
74 h	205-207

Control:

Aqueous 1: 20 mM phosphate buffer, pH: 7.2 and 0.5 M NaCl (10 mL)

Aqueous 2: 20 mM phosphate buffer, pH: 7.2 and 0.5 M Na_2SO_4 (10 mL): 140 mM $NaHCO_3$

Organic Carrier Layer: CH_2Cl_2 (30 mL)

Table S3 Control Experiment

Time	E(mV)
Initial	248-250
21 h	252-253
45 h	220-230
69 h	221-223
93 h	220-224