

A dual host approach to transmembrane transport of salts

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Electronic supplementary information

Vesicle Studies

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (22.32 mg/mL) (Genzyme) was evaporated under reduced pressure to give a thin film. The lipid film was dried under high vacuum for at least 2 hours and rehydrated with phosphate buffer solution (1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts) by vortexing. The lipid suspension was then subjected to nine freeze–thaw cycles and twenty-nine extrusions through a 200 nm polycarbonate nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.) to obtain unilamellar vesicles. The liposomes were passed through a Sephadex (G-50) column to remove unencapsulated dye (eluent; 71mM sodium sulfate buffered to pH 7.2 with 5mM sodium phosphate salts). The vesicles were diluted to 5mL with a solution of 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts to form a stock solution of lipid.

Samples for assay were prepared by diluting lipid stock solution to 3mL (using 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts) to give a solution of 1mM lipid. Fluorescence spectra were obtained using a Varian Cary Eclipse Fluorescence Spectrophotometer. Lucigenin fluorescence was monitored by excitation at 455nm and emission at 506nm for 320s. At $t=10$ s, 100 μ L of MCl (M = Na, K, Rb) stock solution (in 71mM sodium sulfate buffered to pH 7.2 with sodium phosphate) was added such that the concentration of MCl in the external vesicle solution was 100mM. Compounds were added at $t=40$ s as solutions in acetone, to give a 1:50 compound to lipid ratio (2mol%). Lucigenin fluorescence was converted to chloride concentration using the Stern-Volmer constant obtained under assay conditions. Internal chloride concentrations were standardized against the internal chloride concentration at $t=40$ s. Experiments were repeated in triplicate and all traces presented are the average of three trials.

To measure the Stern-Volmer constant, liposomes were prepared using the aforementioned method. Melittin was added at $t=10$ s as a solution in ethanol, to give a 1:500 compound to lipid ratio (0.2mol%). 5 μ L of a stock solution of sodium chloride (in 71mM sodium sulfate, buffered to pH 7.2 with sodium phosphate salts) was added at 20s intervals, such that each addition increased the chloride concentration of the solution by 2mM. A plot of the inverse of the relative fluorescence (F_0/F) against chloride concentration (mM) gave a straight line that could be fitted to the Stern-Volmer equation.

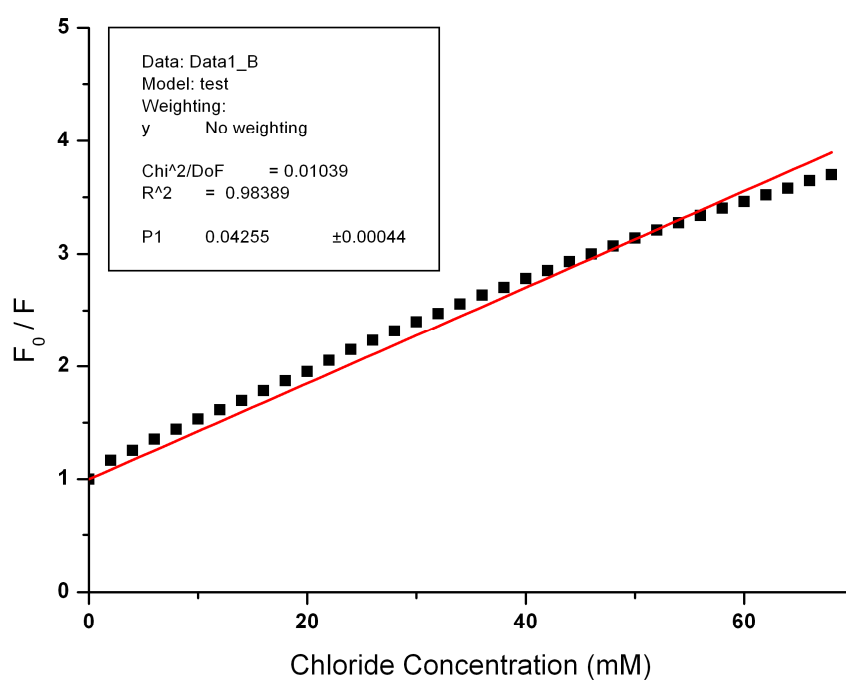


Figure S1: Stern-Volmer plot obtained by adding melittin (0.2mol%) to unilamellar POPC vesicles loaded with 1mM lucigenin and 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in a solution of 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts. 5 μ L of a solution of sodium chloride in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts was added such that each addition increased the total chloride concentration by 2mM.

$$K_{sv} = 42.55 \pm 0.44 \text{ M}^{-1}$$

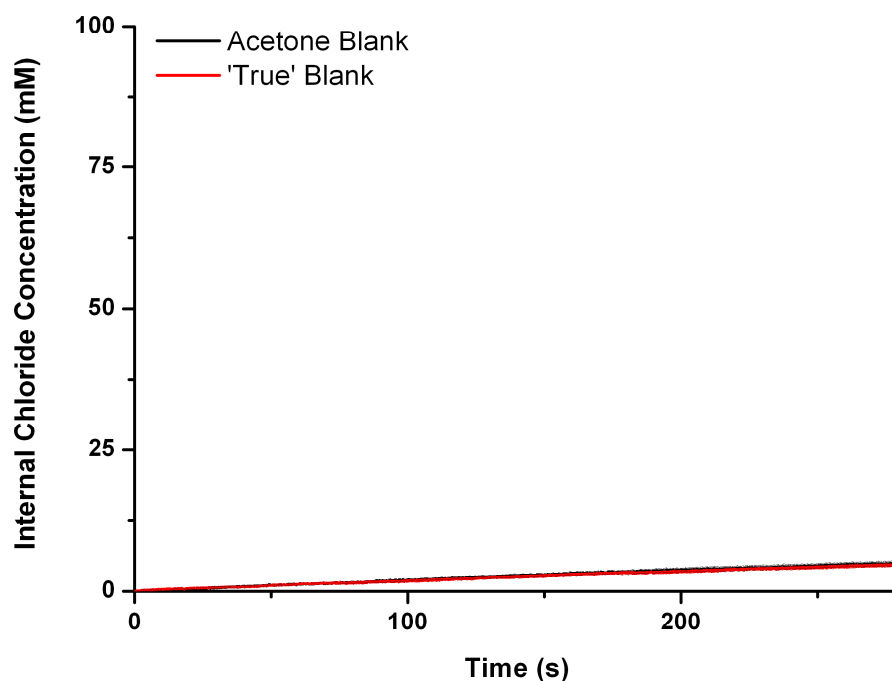


Figure S2 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6 μ L, Acetone Blank) and upon no addition ('True' Blank) following a 100mM NaCl pulse. Error bars included.

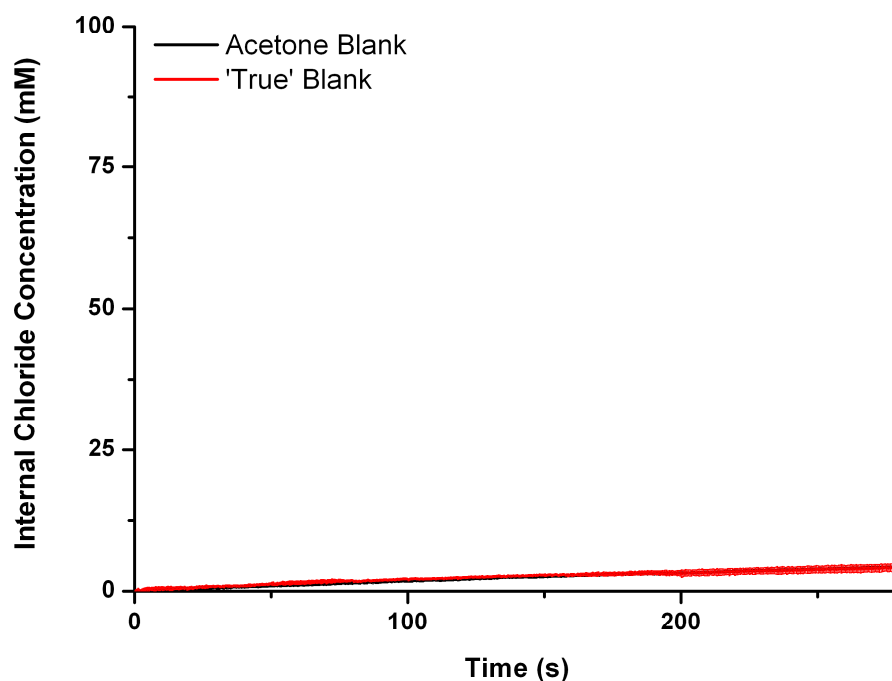


Figure S3 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6 μ L, Acetone Blank) and upon no addition ('True' Blank) following a 100mM KCl pulse. Error bars included.

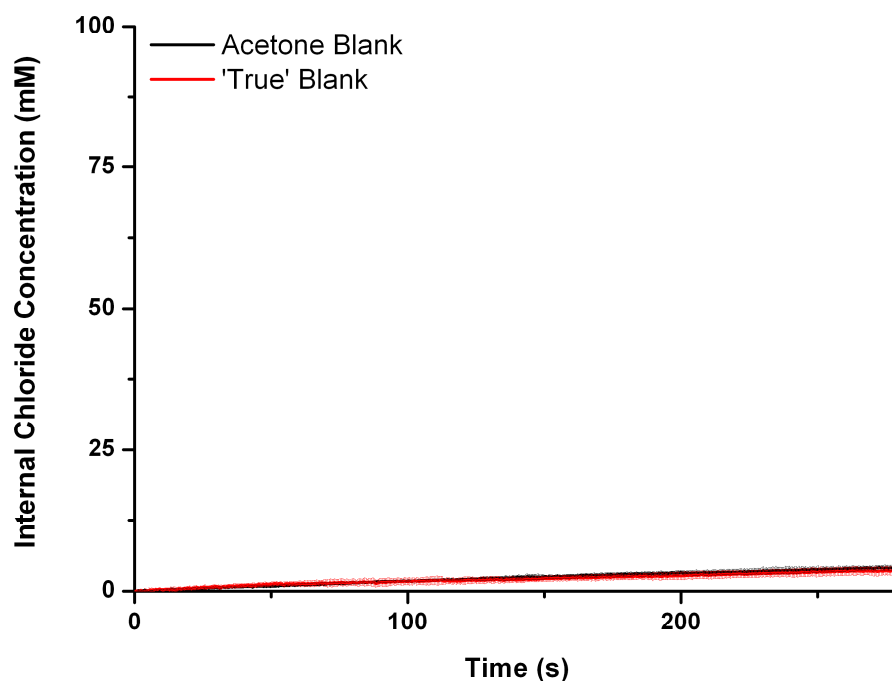


Figure S4 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6 μ L, Acetone Blank) and upon no addition ('True' Blank) following a 100mM RbCl pulse. Error bars included.

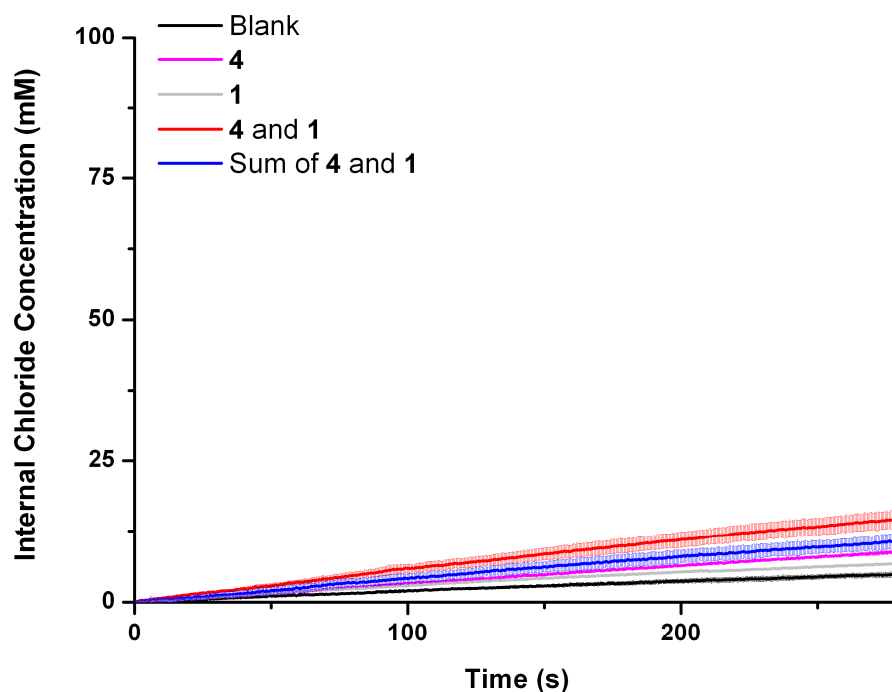


Figure S6 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **1** (2mol%) and both **4** and **1** (2mol% each) following a 100mM NaCl pulse. Error bars included.

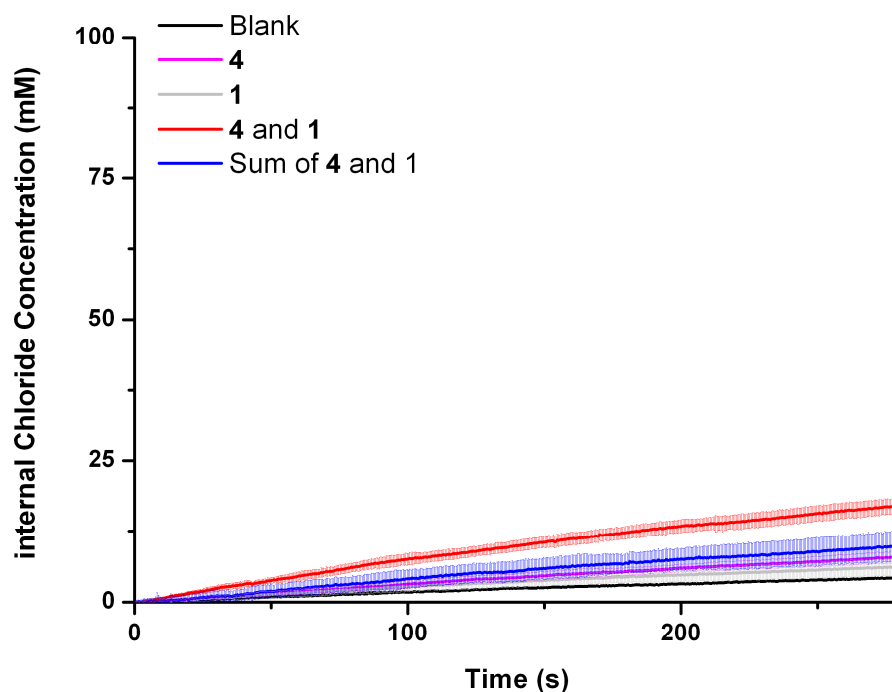


Figure S7 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **1** (2mol%) and both **4** and **1** (2mol% each) following a 100mM KCl pulse. Error bars included.

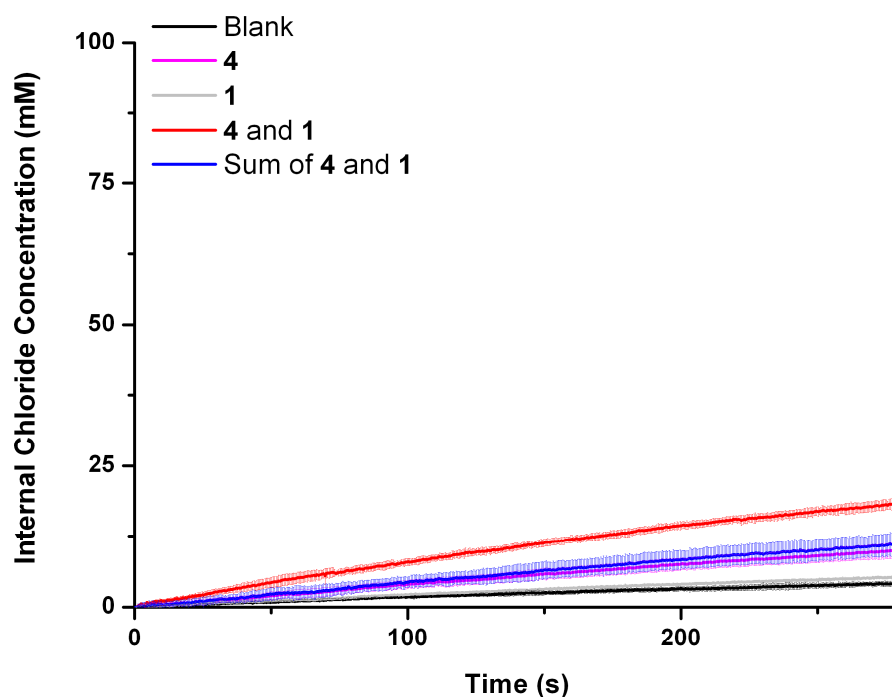


Figure S8 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **1** (2mol%) and both **4** and **1** (2mol% each) following a 100mM RbCl pulse. Error bars included.

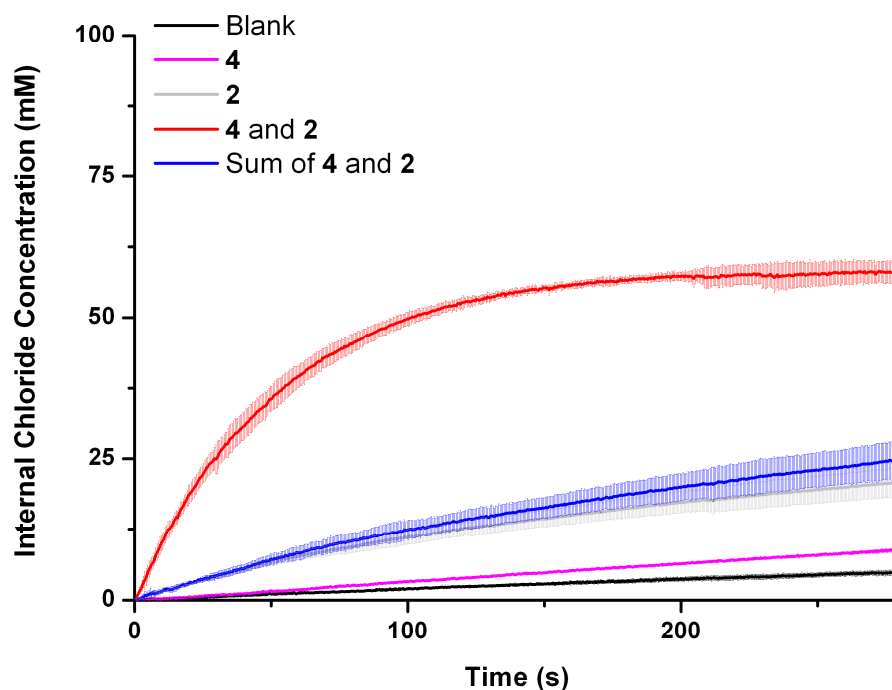


Figure S9 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **2** (2mol%) and both **4** and **2** (2mol% each) following a 100mM NaCl pulse. Error bars included.

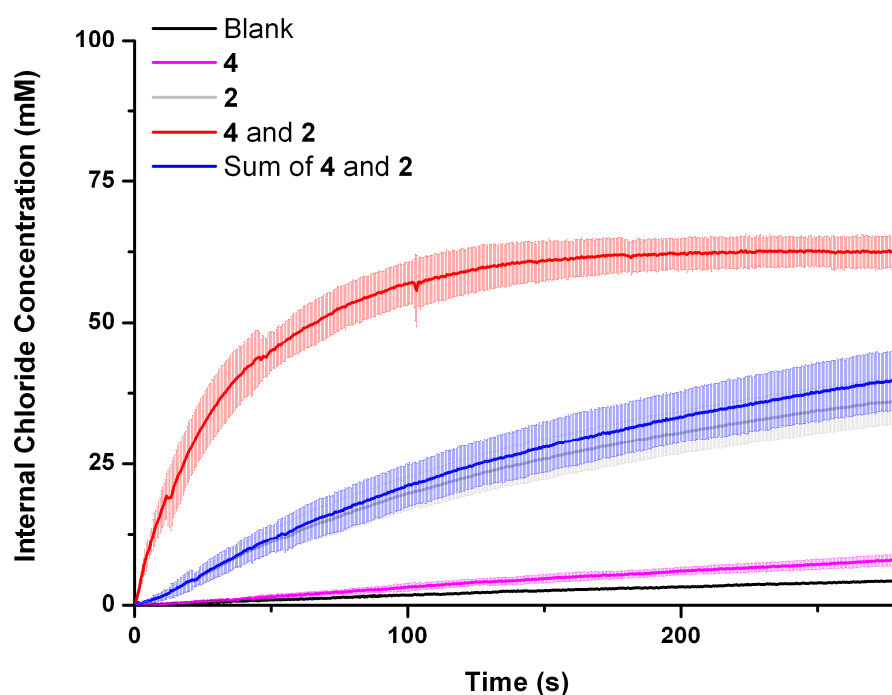


Figure S10 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **2** (2mol%) and both **4** and **2** (2mol% each) following a 100mM KCl pulse. Error bars included.

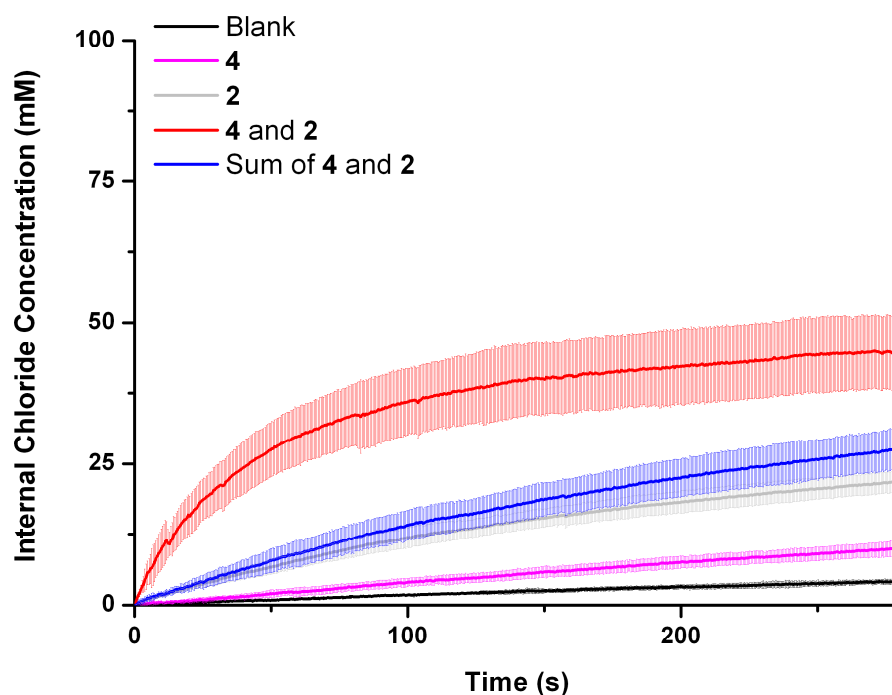


Figure S10 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **2** (2mol%) and both **4** and **2** (2mol% each) following a 100mM RbCl pulse. Error bars included.

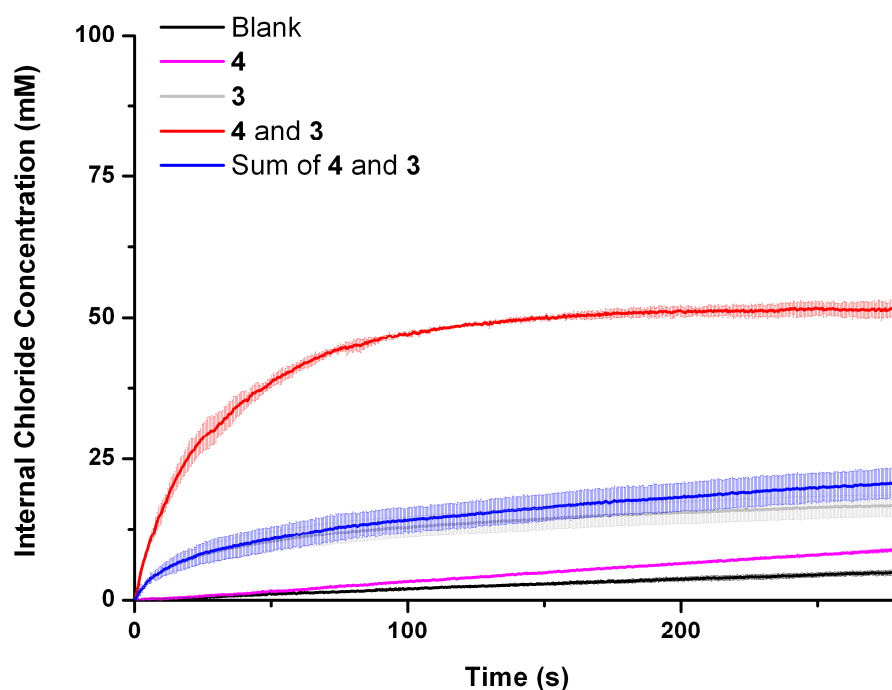


Figure S11 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **3** (2mol%) and both **4** and **3** (2mol% each) following a 100mM NaCl pulse. Error bars included.

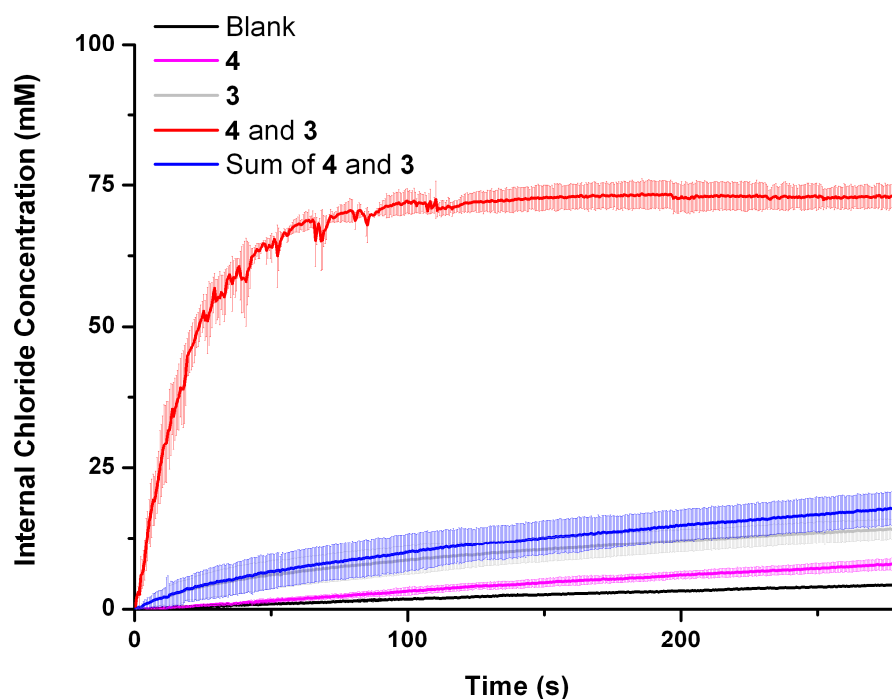


Figure S12 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **3** (2mol%) and both **4** and **3** (2mol% each) following a 100mM KCl pulse. Error bars included.

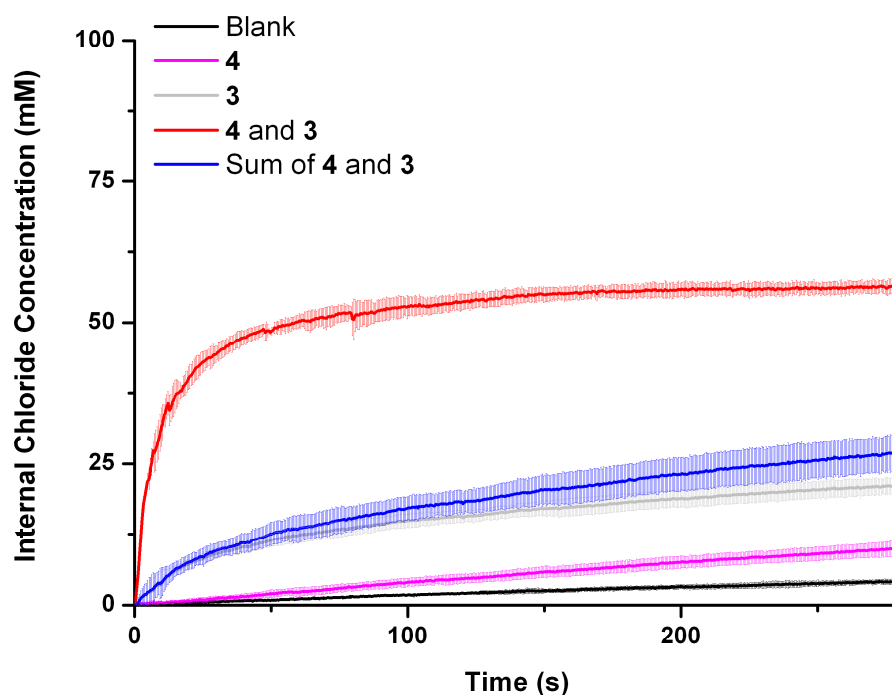


Figure S13 Standardised change in internal chloride concentration of unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%), **3** (2mol%) and both **4** and **3** (2mol% each) following a 100mM RbCl pulse. Error bars included.

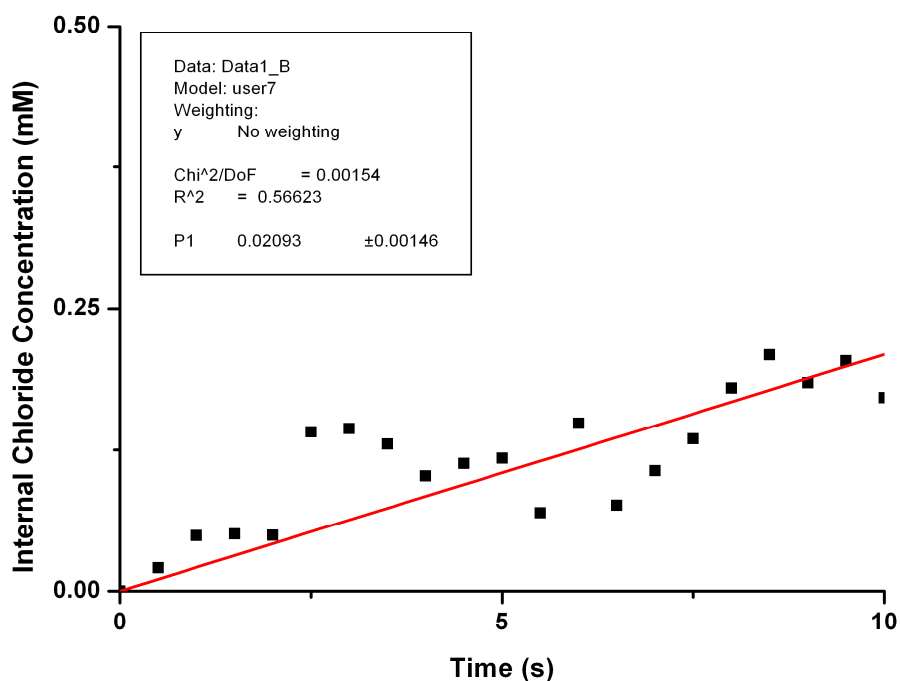


Figure S14 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6 μ L) following a 100mM NaCl pulse.

$$K_i = 0.0209 \pm 0.0015 \text{ mM s}^{-1}$$

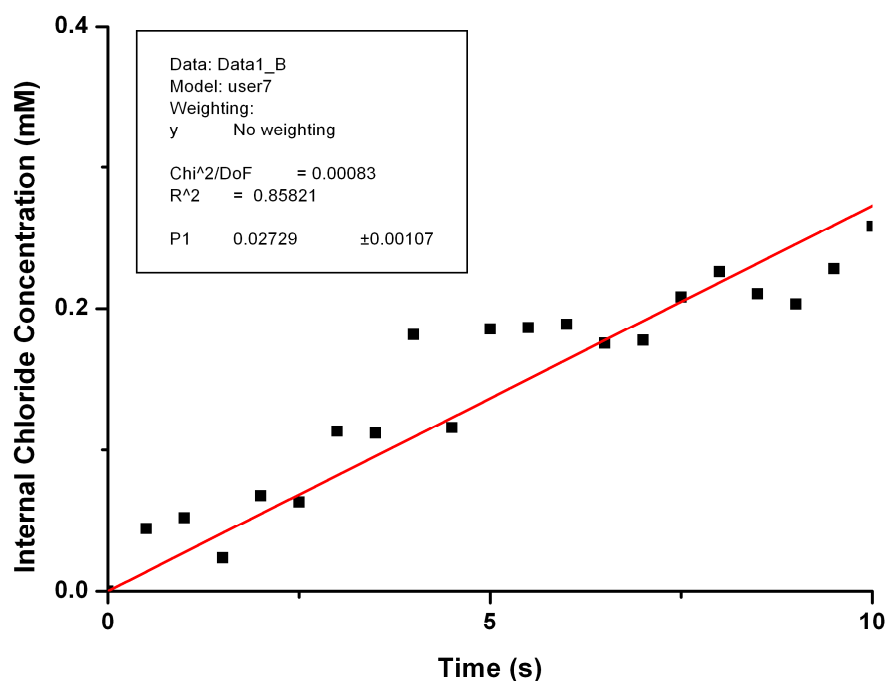


Figure S15 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6μL) following a 100mM KCl pulse.

$$K_i = 0.0273 \pm 0.0011 \text{ mM s}^{-1}$$

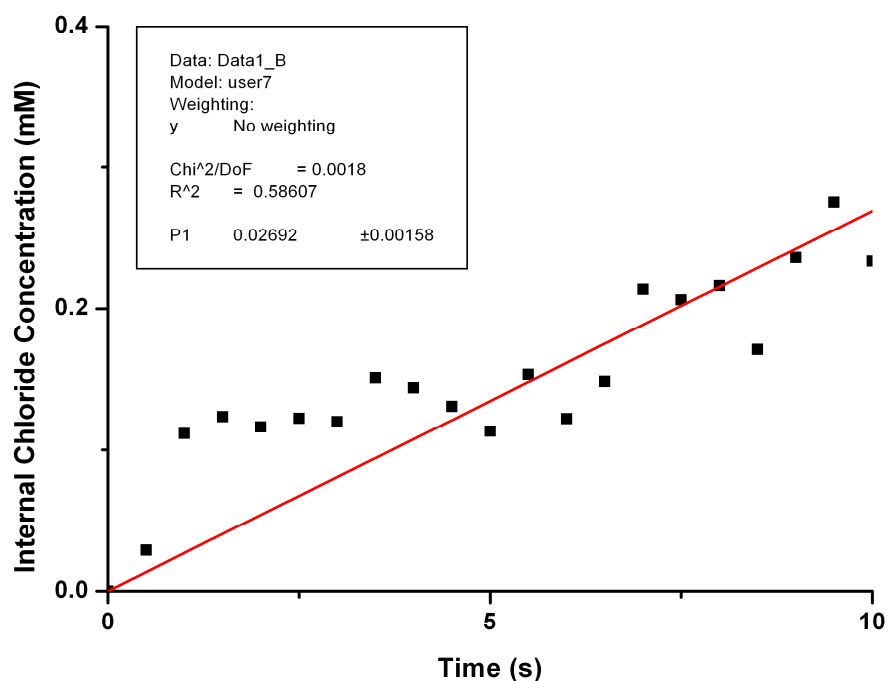


Figure S16 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of acetone (6μL) following a 100mM RbCl pulse.

$$K_i = 0.0269 \pm 0.0016 \text{ mM s}^{-1}$$

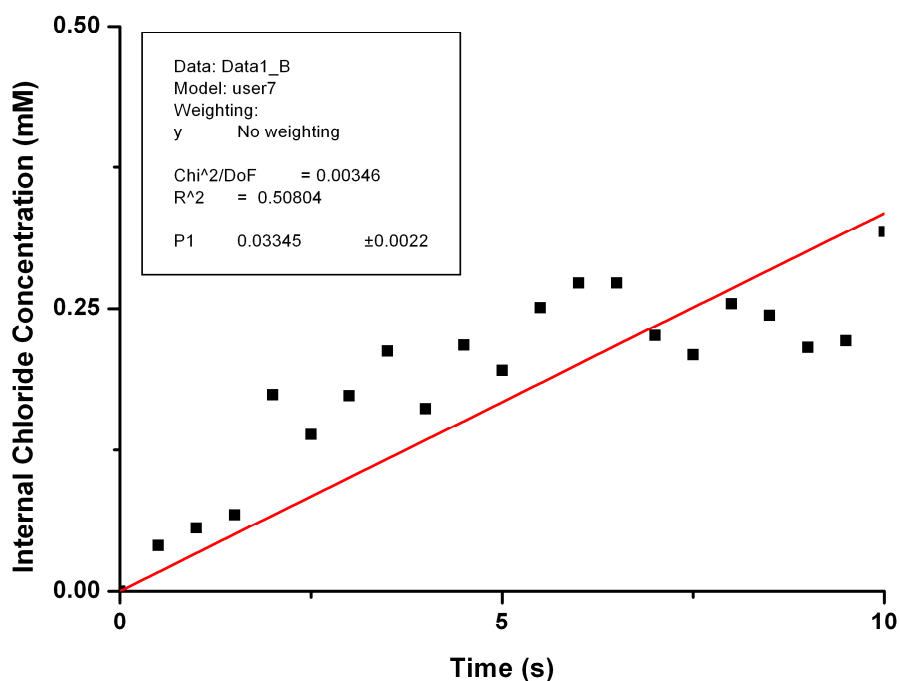


Figure S17 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%) following a 100mM NaCl pulse.

$$K_i = 0.0335 \pm 0.0022 \text{ mM s}^{-1}$$

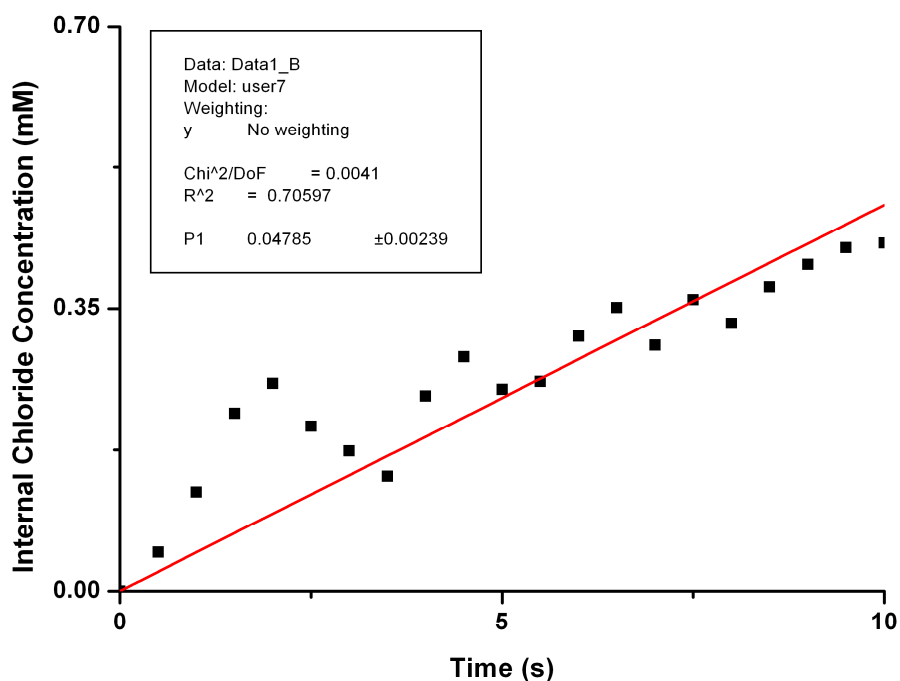


Figure S18 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%) following a 100mM KCl pulse.

$$K_i = 0.0479 \pm 0.0024 \text{ mM s}^{-1}$$

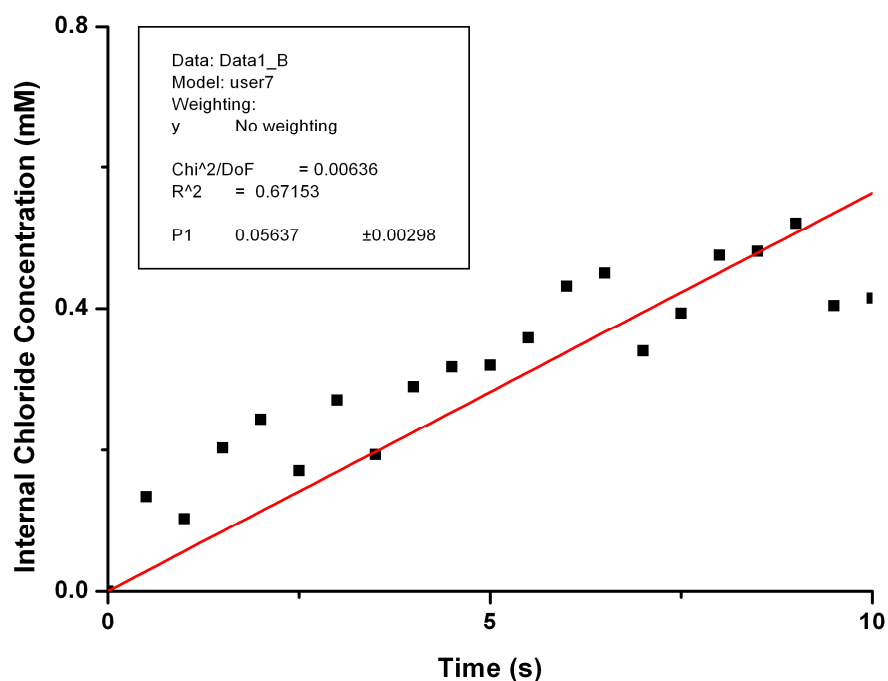


Figure S19 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** (2mol%) following a 100mM RbCl pulse.

$$K_i = 0.0564 \pm 0.0030 \text{ mM s}^{-1}$$

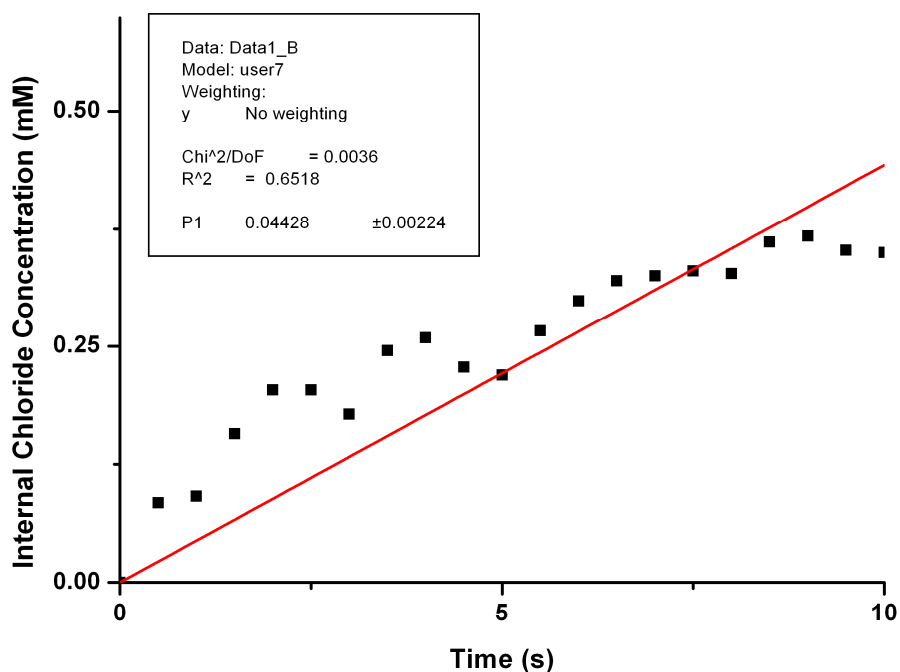


Figure S20 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **1** (2mol%) following a 100mM NaCl pulse.

$$K_i = 0.0443 \pm 0.0023 \text{ mM s}^{-1}$$

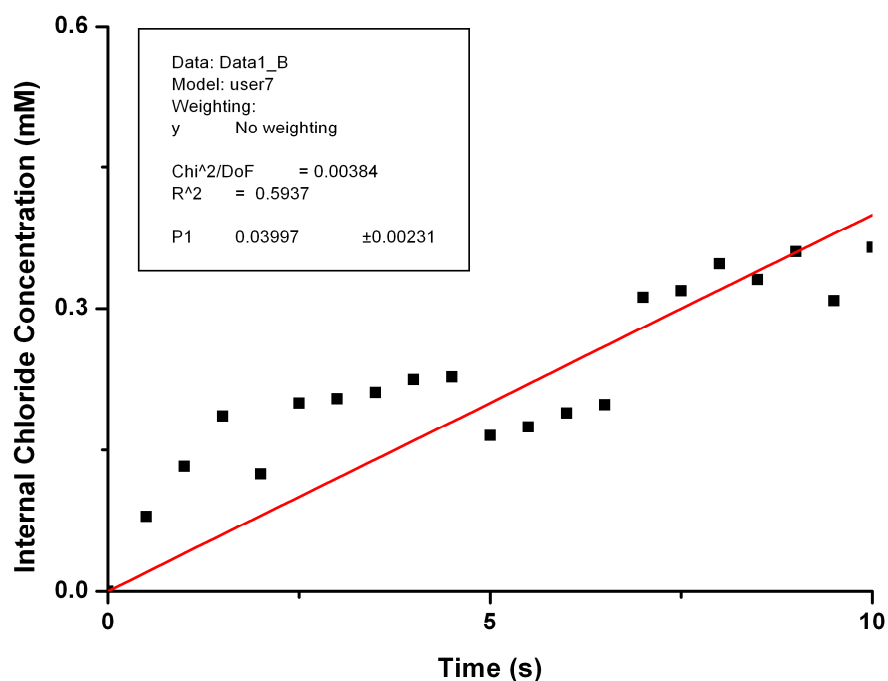


Figure S21 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **1** (2mol%) following a 100mM KCl pulse.

$$K_i = 0.0400 \pm 0.0023 \text{ mM s}^{-1}$$

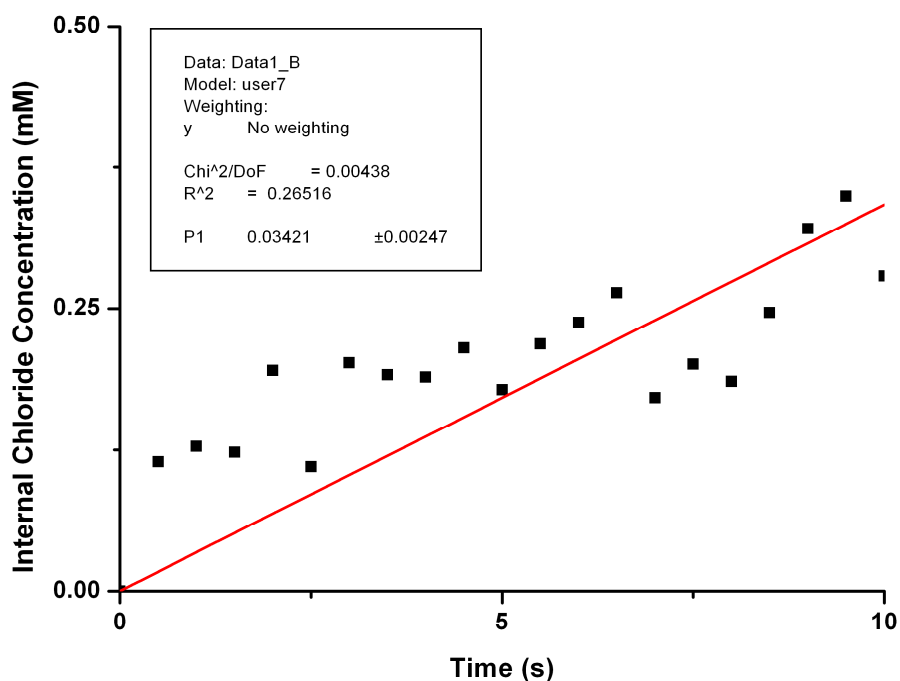


Figure S22 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **1** (2mol%) following a 100mM RbCl pulse.

$$K_i = 0.0342 \pm 0.0025 \text{ mM s}^{-1}$$

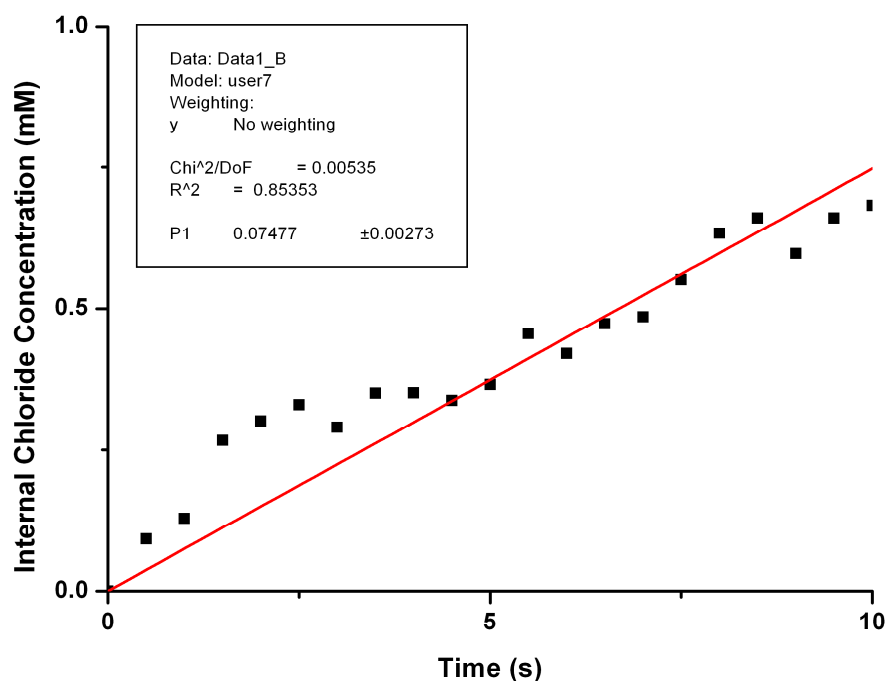


Figure S23 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **1** (2mol% of each) following a 100mM NaCl pulse.

$$K_i = 0.0748 \pm 0.0027 \text{ mM s}^{-1}$$

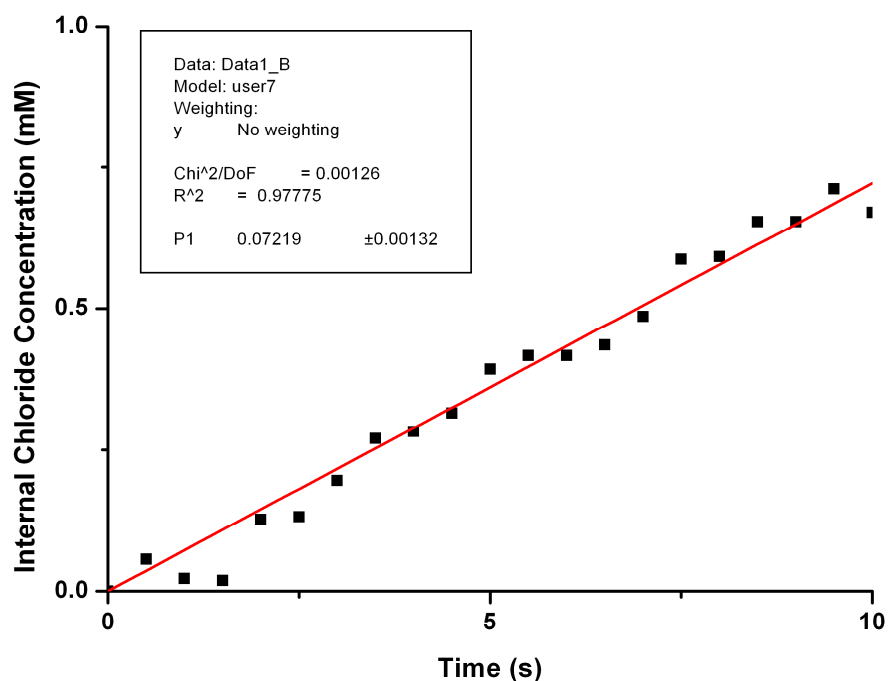


Figure S24 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **1** (2mol% of each) following a 100mM KCl pulse.

$$K_i = 0.0722 \pm 0.0013 \text{ mM s}^{-1}$$

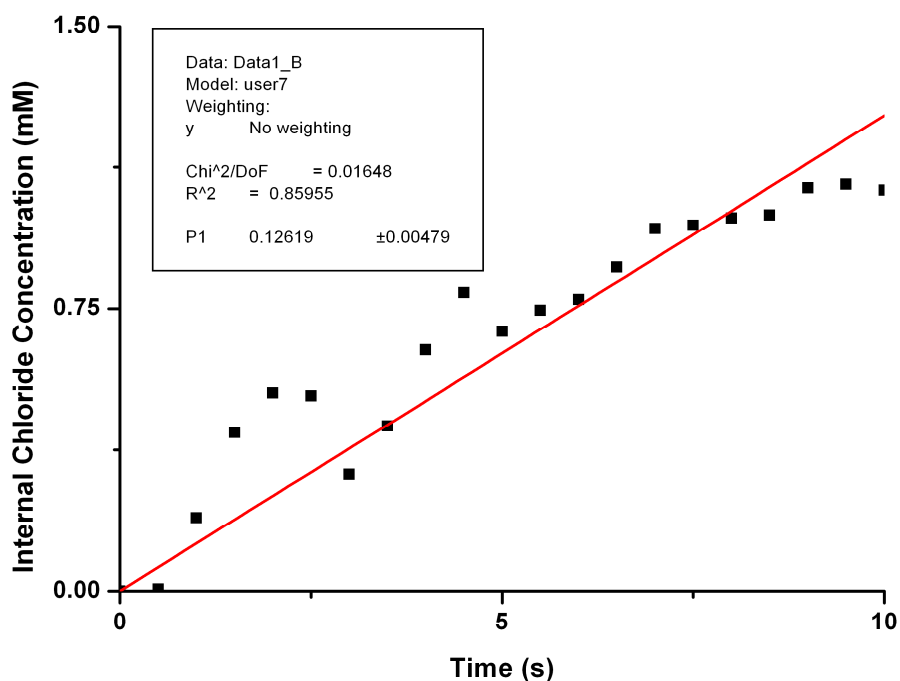


Figure S25 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **1** (2mol% of each) following a 100mM RbCl pulse.

$$K_i = 0.1262 \pm 0.0048 \text{ mM s}^{-1}$$

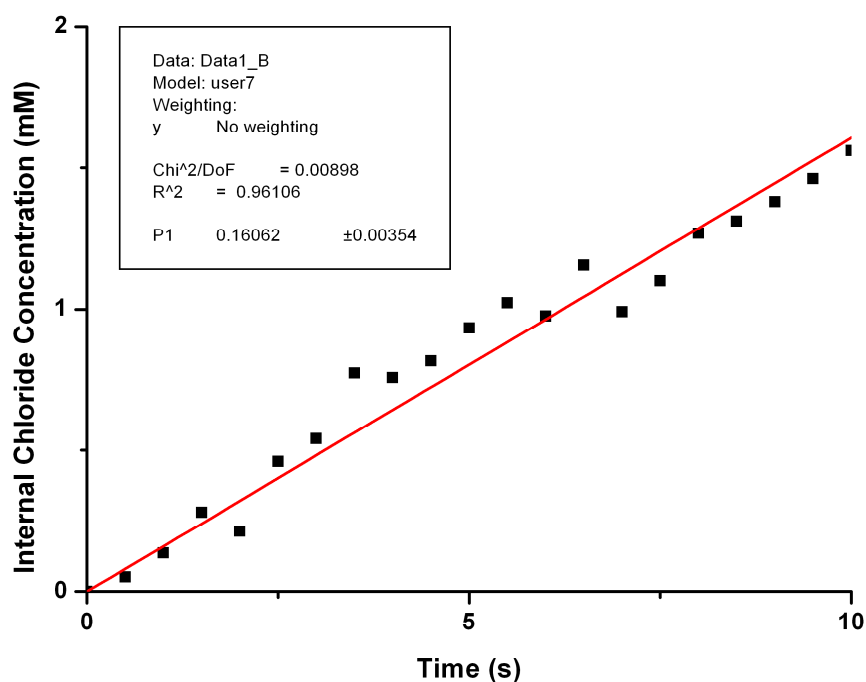


Figure S26 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **2** (2mol%) following a 100mM NaCl pulse.

$$K_i = 0.1606 \pm 0.0035 \text{ mM s}^{-1}$$

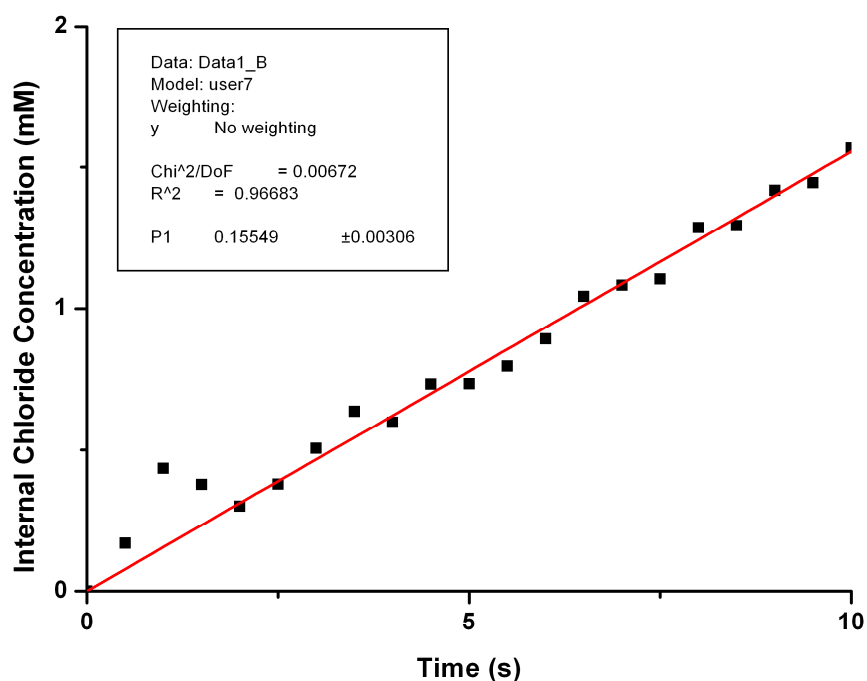


Figure S27 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **2** (2mol%) following a 100mM KCl pulse.

$$K_i = 0.1555 \pm 0.0031 \text{ mM s}^{-1}$$

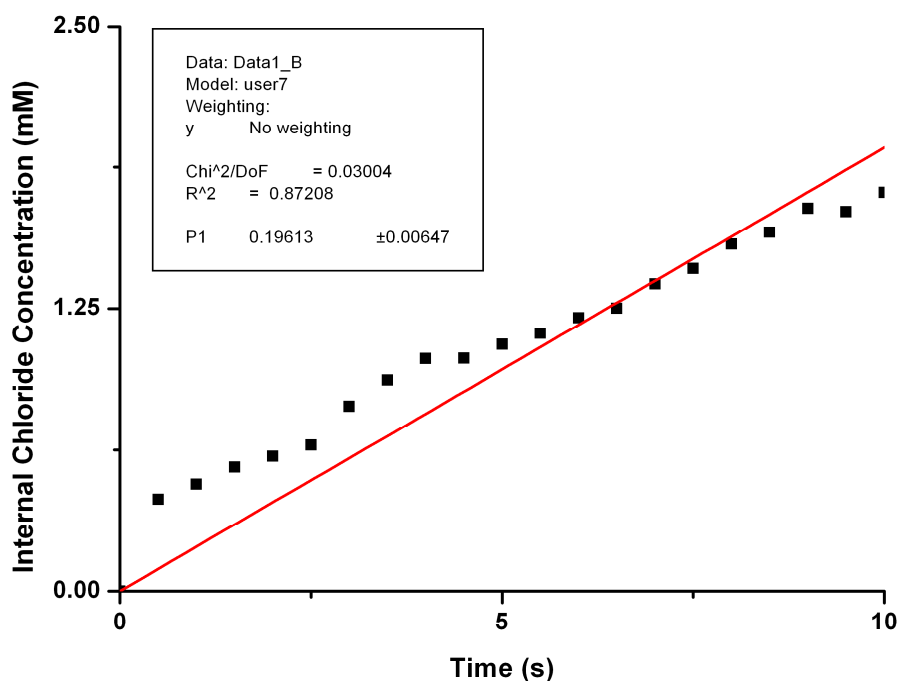


Figure S28 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **2** (2mol%) following a 100mM RbCl pulse.

$$K_i = 0.1961 \pm 0.0055 \text{ mM s}^{-1}$$

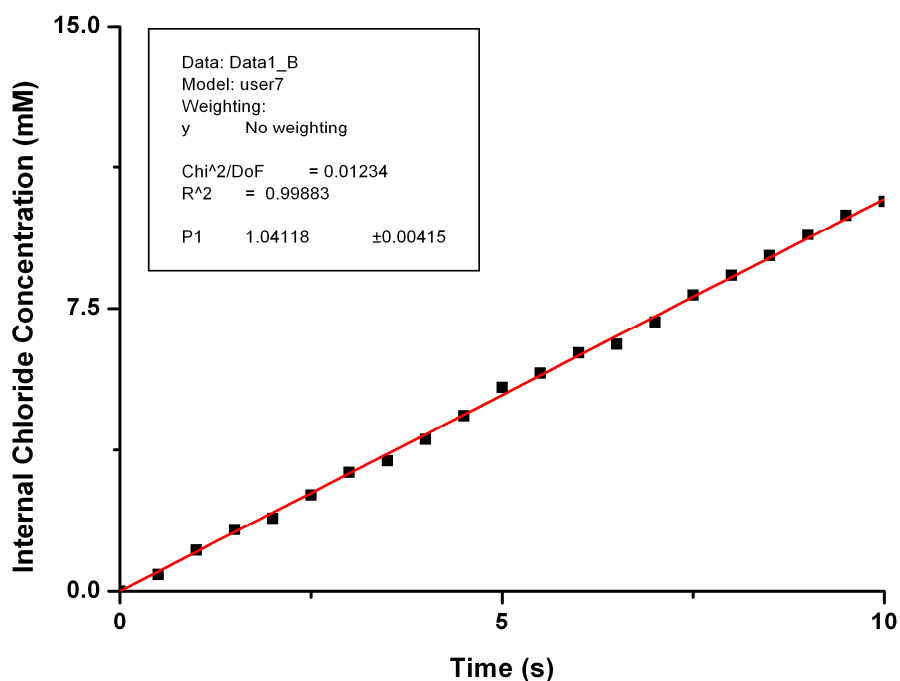


Figure S29 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **2** (2mol% of each) following a 100mM NaCl pulse.

$$K_i = 1.0412 \pm 0.0042 \text{ mM s}^{-1}$$

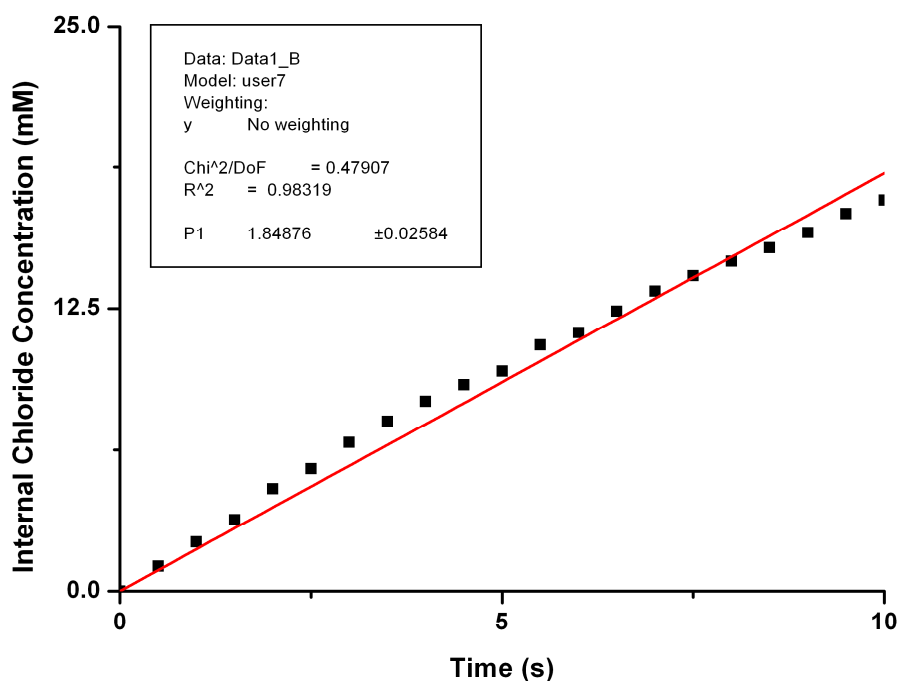


Figure S30 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **2** (2mol% of each) following a 100mM KCl pulse.

$$K_i = 1.8488 \pm 0.0258 \text{ mM s}^{-1}$$

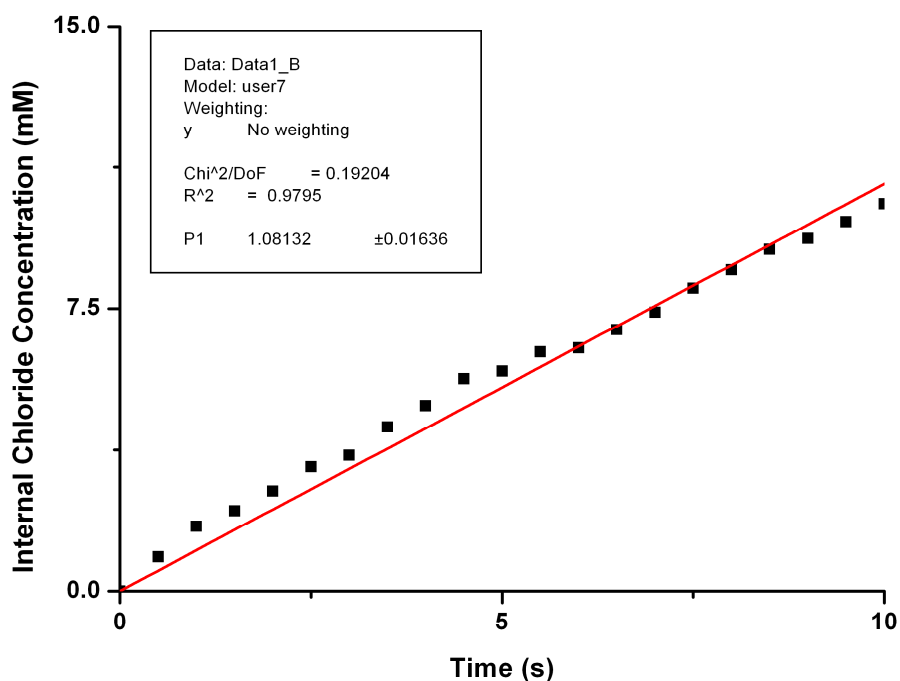


Figure S31 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **2** (2mol% of each) following a 100mM RbCl pulse.

$$K_i = 1.0813 \pm 0.0164 \text{ mM s}^{-1}$$

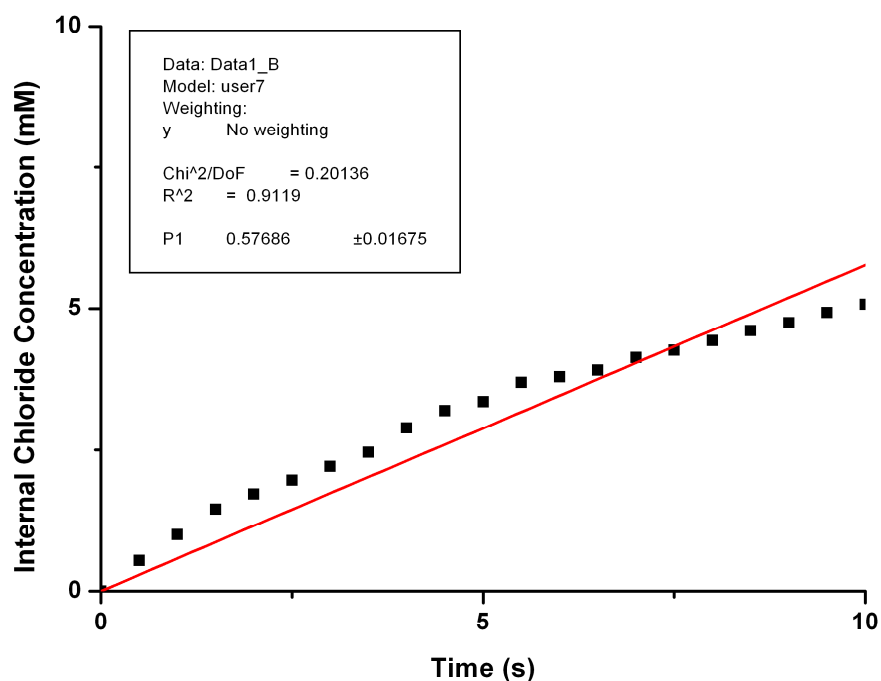


Figure S32 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **3** (2mol%) following a 100mM NaCl pulse.

$$K_i = 0.5769 \pm 0.0168 \text{ mM s}^{-1}$$

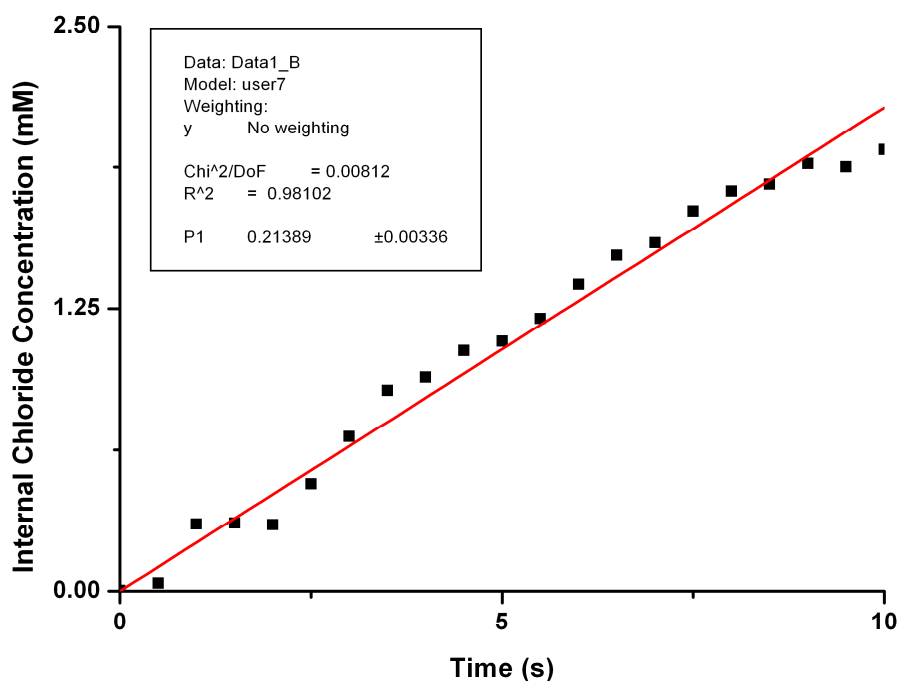


Figure S33 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **3** (2mol%) following a 100mM KCl pulse.

$$K_i = 0.2139 \pm 0.0034 \text{ mM s}^{-1}$$

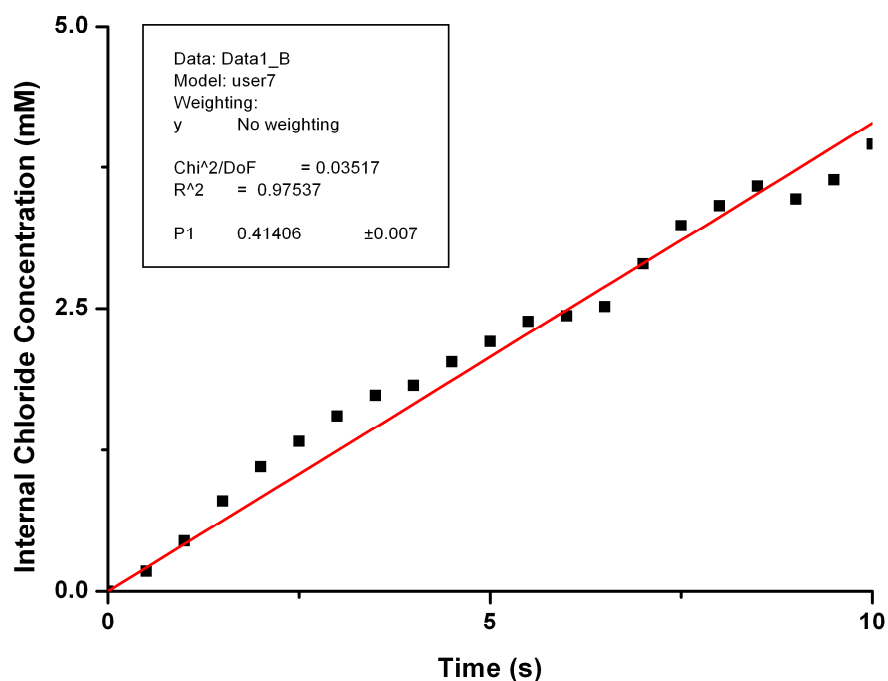


Figure S34 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **3** (2mol%) following a 100mM RbCl pulse.

$$K_i = 0.4141 \pm 0.0070 \text{ mM s}^{-1}$$

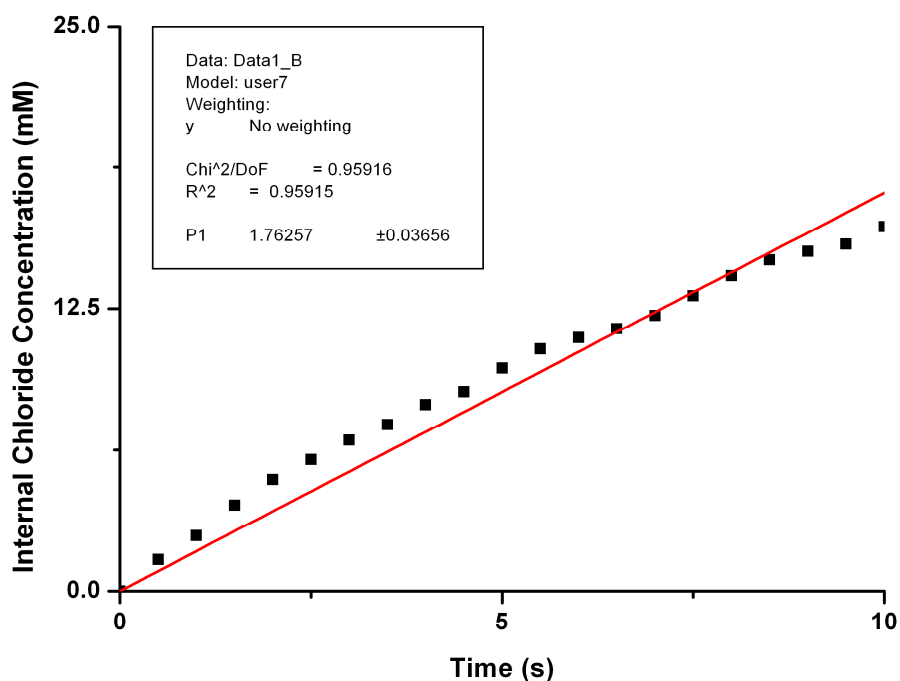


Figure S35 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **3** (2mol% of each) following a 100mM NaCl pulse.

$$K_i = 1.7626 \pm 0.0366 \text{ mM s}^{-1}$$

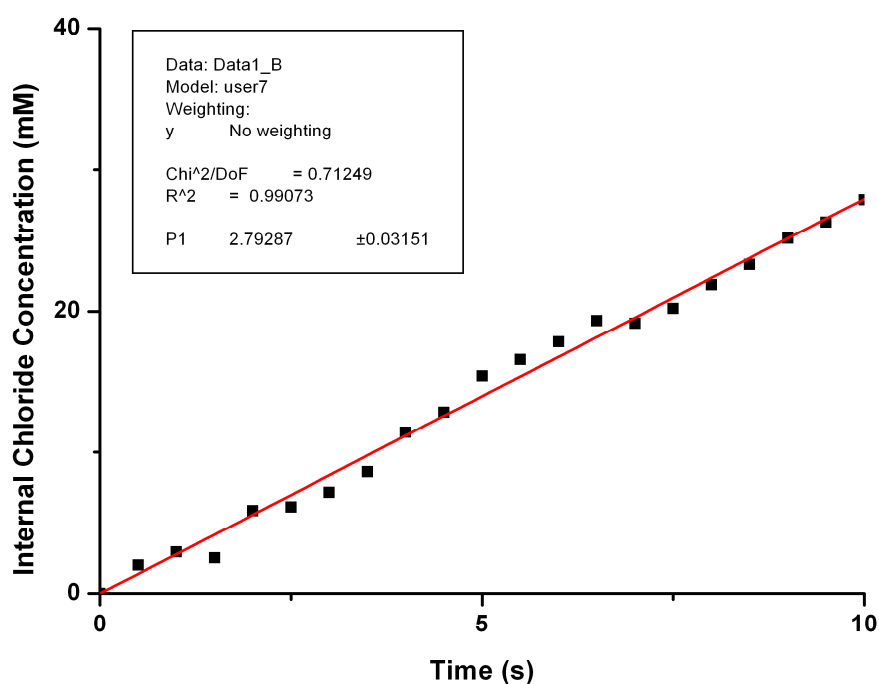


Figure S36 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **3** (2mol% of each) following a 100mM KCl pulse.

$$K_i = 2.7929 \pm 0.0315 \text{ mM s}^{-1}$$

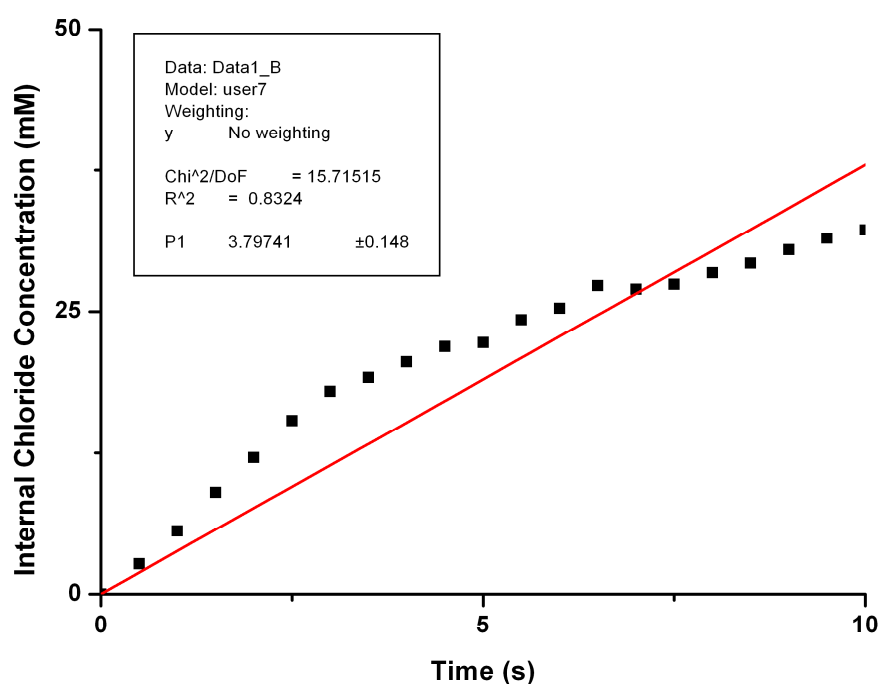


Figure S37 Initial rate constant determination for unilamellar POPC vesicles containing 1mM lucigenin, 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts and suspended in 71mM sodium sulfate, buffered to pH 7.2 with 5mM sodium phosphate salts, upon addition of an acetone solution of **4** and **3** (2mol% of each) following a 100mM RbCl pulse.

$$K_i = 3.7974 \pm 0.1480 \text{ mM s}^{-1}$$