Supplementary Information

Experimental

Materials

Unless otherwise stated, all materials and solvents are from Sigma-Aldrich (Gillingham, Dorset, UK). NMR spectra were recorded on a Bruker AM-360 instrument at 360 and 90 MHz for ^{1}H and ^{13}C respectively. 1,3,5-Trimethoxyhexa(t-butyl)calix[6]arene was prepared from t-butylcalix[6]arene according to the literature.[1] Chemical shifts were referenced to tetramethylsilane (TMS). ESI mass spectra were recorded on a Bruker micrOTOF instrument operating in the positive mode.

Tritontosylate (**1**)

Triton-X100® (15.18 mL, 16.2 g, 0.0025 mol) was dissolved in THF (15 mL) and distilled water (5 mL) to which an aqueous solution of NaOH (1.42 g in 5.5 mL) was added. The mixture was stirred in an ice bath until the solution temperature fell below 5 ºC. *p*-Toluenesufonyl chloride (5.33 g, 0.027 mol) in THF (10 mL) was added to the stirred solution at a rate that kept the temperature below 5 ºC. Once the addition was complete the solution was left to stir for a further 30 minutes whereupon it was poured onto 20 g of ice and 10 mL of water, then added to toluene (30 mL). Crude **1** was extracted into toluene and dried over calcium chloride. Following filtration, solvent was removed under vacuum to give **1** as a colourless oil. Yield: 10.2 g (51%); ^{1}H NMR ($CDCl_3$) δ 7.79 (d, 4H, *J* = 7.9, tosylAr*H*), 7.32 (d, 4H, *J* = 7.9, tosylAr*H*), 7.25 (d, 4H, *J* = 8.6, Ar*H*), 6.81 (d, 4H, *J* = 8.6, Ar*H*), 4.14 (t, 2H, tosylArCH_2), 4.10 (t, 2H, ArCH_2), 3.83 (t, 2H, tosylArCH$_2$CH_2), 3.71-3.56 (m, 34H, OCH_2CH_2), 2.43 (s, 3H, tosylCH_3), 1.69 (s, 2H, CH_2CH$_3$), 1.33 (s, 6H, ArC(CH_3)$_2$), 0.70 (s, 9H, C(CH_3)$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 156.4, 144.7, 142.3, 137.8, 133.1, 129.8, 129.0, 128.0 ,127.0, 125.3, 113.8, 72.5, 71.0, 70.6, 70.3, 69.8, 69.2, 68.7, 67.3, 57.0, 37.9, 32.3, 31.8, 21.6; ESI HRMS *m/z* found: 823.4467 (M + Na^+), calculated: 823.4279.

t-Butylcalix[4]arene(OH)$_2$(Triton)$_2$ (**2**)

t-Butylcalix[4]arene (1.28 g, 1.96mmol) was dissolved in anhydrous acetonitrile (40mL). Triton tosylate, **1**, (3.93 g, 4.9 mmol) and K_2CO_3 (0.54 g, 4 mmol) were added and the mixture refluxed under nitrogen for 72 h. The cooled mixture was filtered to remove solid byproducts and the solvent was removed under vacuum to give **2** as colorless, viscous oil. Yield: 1.86 g (50%); ^{1}H NMR ($CDCl_3$) δ 7.74 (s, 2H, ArO*H*),

7.25 (d, 4H, *J* = 8.6, tritonAr*H*), 7.03 (s, 4H, Ar*H*), 6.95 (s, 4H, Ar*H*), 6.82 (d, 4H, *J* = 8.3, tritonAr*H*), 4.38 (d, 4H, *J* = 13.0, ArCH_2Ar), 4.11 (t, 4H, ArOCH_2), 3.82 (m, 2H, ArOCH$_2$CH_2), 3.71-3.57 (m, 68H, OCH_2CH_2), 3.30 (d, 4H, *J* = 12.6, ArCH_2Ar), 1.69 (s, 4H, CH_2CH$_3$), 1.33 (s, 12H, ArC(CH_3)$_2$), 1.25 (s, 18H, ArC(CH_3)), 1.08 (s, 18H, ArC(CH_3)), 0.70 (s, 18H, C(CH_3)$_2$CH$_2$CH_3); ^{13}C NMR (CDCl$_3$) δ 156.4, 150.6, 149.9, 146.7, 142.3, 141.2, 132.6, 129.8, 127.9, 127.7, 127.0, 125.5, 125.0, 113.8, 71.0, 70.7, 70.3, 69.8, 69.2, 67.3, 57.0, 37.9, 33.9, 32.3, 31.8, 31.7; ESI HRMS m/z found: 1929.2877 (M + Na$^+$), calculated: 1929.2486.

t-Butylcalix[6]arene(OMe)$_3$(Triton)$_3$ (**3**)

1,3,5-Trimethoxyhexa(t-butyl)calix[6]arene (1.99 g, 1.96 mmol) was dissolved in anhydrous acetonitrile (40 mL). Triton tosylate, **1**, (5.9 g, 7.35 mmol) and K_2CO_3 (0.54 g, 4 mmol) were added and the mixture refluxed under nitrogen for 72 h. The cooled mixture was filtered to remove solid byproducts and the solvent was removed under vacuum to give **3** as a colourless, viscous oil. Yield: 2.42g (42%); ^{1}H NMR (CDCl$_3$) δ 7.24 (d, 6H, *J* = 8.6, tritonAr*H*), 7.01 (s, 6H, Ar*H*), 6.90 (s, 6H, Ar*H*), 6.82 (d, 6H, *J* = 8.6, tritonAr*H*), 4.10 (t, 6H, ArOCH_2), 3.87 (m, 6H, ArCH_2Ar), 3.85 (t, 6H, ArOCH$_2$CH_2), 3.70 (s, 9H, OCH_3), 3.55-3.70 (m, 108H, OCH_2CH_2), 3.50 (m, 6H, ArCH_2Ar), 1.69 (s, 6H, CH_2CH$_3$), 1.33 (s, 18H, ArC(CH_3)$_2$), 1.22 (s, 27H, ArC(CH_3)), 1.08 (s, 27H, ArC(CH_3)), 0.71 (s, 27H, C(CH_3)$_2$CH$_2$CH_3); ^{13}C NMR (CDCl$_3$) δ 156.5, 153.3, 152.4, 149.8, 149.4, 146.8, 146.6, 142.4, 132.3, 126.7, 126.0, 125.7, 124.9, 113.9, 70.6, 69.9, 67.4, 61.5, 57.1, 38.0, 33.9, 32.3, 31.8, 31.7; ESI HRMS *m/z* found: M^{3+} 966.7254, calculated: 966.6432.

Molecular modelling

A simplified model of calixarene, **3**, 5,11,17,23,29,35-hexa-t-butyl-37,39,41-trimethoxy-38,40,42-triethoxycalix[6]arene, was constructed using the Spartan '06 graphical interface. Pseudo C_{3v} symmetry was imposed by constraining alternating aromatic rings to 60º. The model was geometry optimized using the MMFF parameters to give a conformer in which three t-butyl groups interlocked above the central cavity and a second, more symmetrical conformer with a central void. Single point energy calculations (PM3(d)) gave Hº of -593.2 kJ mol^{-1} for the former and -601.8 kJ mol^{-1} for the latter. The overall ΔHº is therefore 8.6 kJ mol^{-1} in favour of the 'open' conformer.[2]

Crystallographic evidence for *endo*/*exo* positions of lower rim substituents

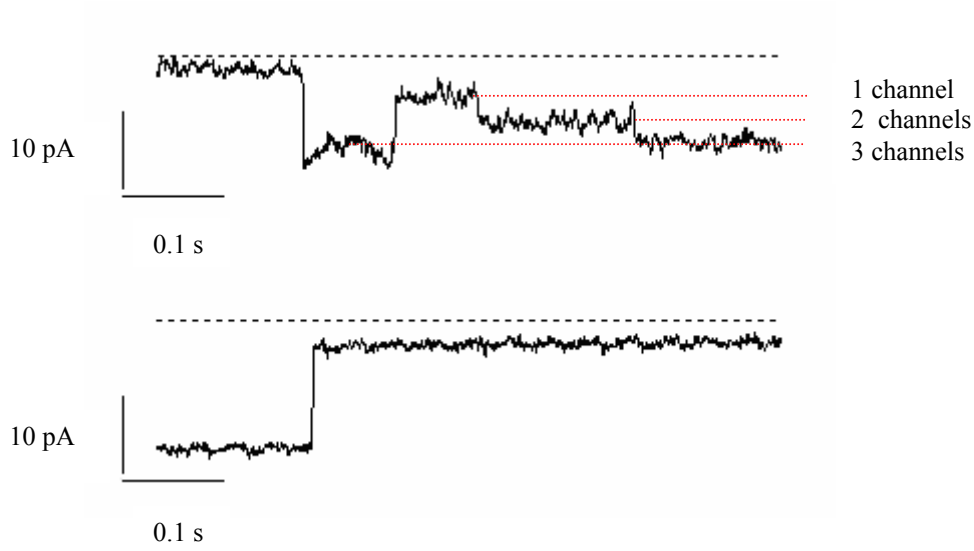
A search of the Cambridge Structural Database reveals 41 1,3,5-trimethoxycalix[6]arene cone conformers in which the 2,4,6-positions have larger alkyl substituents. Of these, 17 (AJUTEE, FAWWAG, FAWWEG, HIVRIN, JEMJUH, PEDNIW, PEDNOC, PEDNUI, PZWXIZ, QURGIT, QURGIT01, QURGUF, VEQJUX, VEQKAE, WUDZIE, WUFBEE, YEGDIY) have no metal atoms coordinated within the lower, phenolic cavity and have methyl groups blocking the cavity. The remaining structures all exhibit *exo* geometries for all three methyl groups - three (KALYEC, KALYIG, SIQWIY) contain solvent but no metal, one (LUFGUO) is a self inclusion complex, one (XAGRUS) is a rotaxane, and the remainder (CANXUL, FAYWUY, FOQIR, FOQWOX, HETPOM, PAYPAH, QUZFAM, QURGOZ, SAQKIF, UMADAW, WUDZOK, WUDZUQ, WUFBEG, WUFBUU, WUFCAB, WUFCEF, WUFCIJ, XAFFOX, XAFFUF) contain coordinated metals, usually with a solvent molecule coordinated to the metal, inside the calixarene cavity. All three available 1,3,5-triethoxycalix[6]arene cone conformer structures (QOZFEQ, QUMLUF, WUFBAA) incorporate coordinated metals and all the ethyl groups are *exo* to the cavity. In every example where a metal coordinates to the lower rim of the calixarene the 1,3,5-substituents (methyl or ethyl) adopt a position *exo* to the cavity.

Planar bilayer experiments

Planar lipid bilayer recordings were carried out as described previously.[3] Pure synthetic lipids (Avanti Polar Lipids, Birmingham, AL USA) were dispersed in chloroform and stored at –70^{o}C under nitrogen. Lipid bilayers were formed from a dispersion of 15 mg/ml 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE) and 15 mg/ml 1-palmitoyl-2-oleoyl phosphatidylserine (POPS) in *n*-decane, which was drawn across a 0.25 mm diameter hole in a polystyrene cup separating two solution filled chambers, designated *cis* and *trans*. The *cis* chamber (to which the calixarenes were added) was held at ground, and the *trans* chamber was clamped at -50mV using a Warner PC501A patch clamp amplifier equipped with a 10GB (10GΩ) bilayer headstage (Warner Instruments). The sign of the membrane potential refers to the *trans* chamber, and currents are defined as positive when cations flow from *trans* to *cis*. Transmembrane currents were low pass filtered at 500Hz (4 pole Bessel) digitised at 10 kHz and recorded directly to disk via a CED Micro 1401 Mark II AD interface. Membrane capacitance was measured by differentiating a triangular wave input of 0.2 kHz. Only bilayers that had a resting

conductance of less than 10 pS and an initial capacitance of at least 150 pF were used. Unless other wise stated bilayers were bathed in symmetrical solutions containing 150 mM NaCl; 10mM HEPES; 1 mM EGTA; 1.05 mM $CaCl_2$; 1mM $MgCl_2$; 50 μM free Calcium. All recordings were made at room temperature. Recordings were analysed off line using win EDR v2.3.9 software (Strathclyde electrophysiological software). Maximum current amplitudes were determined from the peaks of Gaussian functions fitted to amplitude histograms.

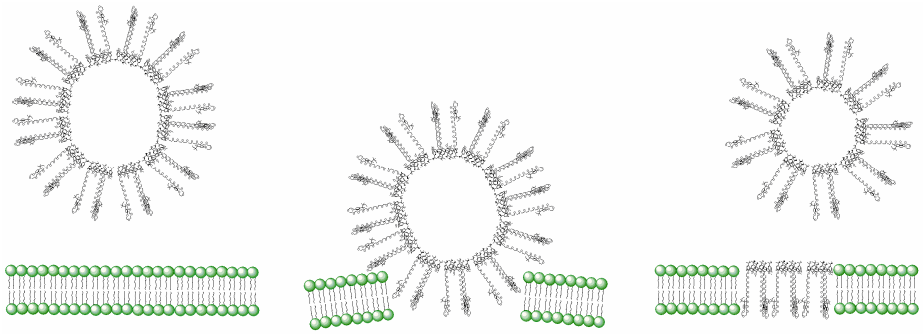
Two sections from the same recording (04/07/07) below illustrate typical features observed.



Simultaneous opening of 3 channels followed by loss of two channels then sequential opening of two channels (upper trace), simultaneous loss of 4 channels (lower trace).

Proposed mode of calixarene insertion in the planar bilayer

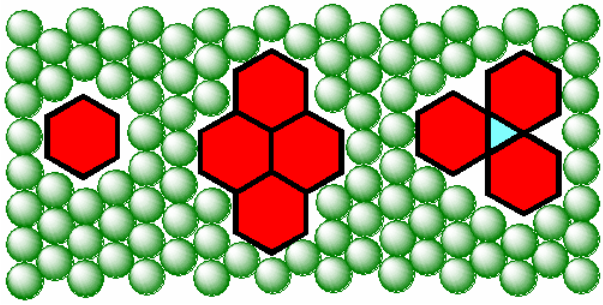
The calixarene derivative has a lipophilic head group and amphiphilic 'tails' so we expect molecules to form micelles at concentrations above the cmc, interacting with each other predominantly through the hydrophobic effect. At 64 μM the solution used is visibly opalescent, indicative of micelle formation, and we propose that a small portion of a micelle fuses with the bilayer thereby inserting several molecules to form a multichannel. This is consistent with the well-known fusion method of introducing channel-forming proteins to bilayers.[4] A section through a micelle and bilayer is shown to illustrate this.



A micelle, composed of the derivatized calixarene with amphiphilic polyethers on its exterior, approaches the phospholipid bilayer (left). A portion of the micelle inserts in the bilayer (centre) and is left *in situ* when the micelle moves away from the bilayer (right).

Proposed mode of multichannel conductance

The cartoon below illustrates a single molecule inserting to give a current of I (left), four molecules inserting, interacting primarily through hydrophobic effects, giving a current of 4 I (centre), and a group of three molecules with an interstitial channel (right). The latter arrangement would result in a current oscillating between 3 I and 4 I, depending on the permittivity of the interstitial channel, denoted by the blue triangle.



A similar interpretation has been made by Fyles[5] to explain the large step changes in conductivity observed for channel-forming oligoesters.[6]

1. H. Otsuka, K. Araki and S. Shinkai, *Tetrahedron*, 1995, **51**, 8757.
2. Spartan `06, Wavefunction Inc., 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612
3. S. P. Hardy, C. Richie, M. C. Allen, R. H. Ashley and P. E. Granum, *BBA Biomembranes*, 2001, **1515**, 38.
4. *Ion Channels and Disease*, F. M. Ashcroft, Academic Press, 2000, p. 53.
5. T. M. Fyles, *Structure-activity studies on oligoester ion channels*, presented at the 2nd International Symposium on Macrocyclic and Supramolecular Chemistry, Salice Terme, Italy, 24th-28th June 2007.
6. T. M. Fyles, C.-W. Hu and H. Luong, *J. Org. Chem.*, 2006, **71**, 8545.