Supplementary Material (ESI) for Chemical Communications

Supporting Information for Apparent Power Law Distribution of Open Durations in Cyclodextrin Ion Channels

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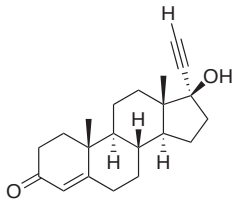
Contents

1 Synthesis

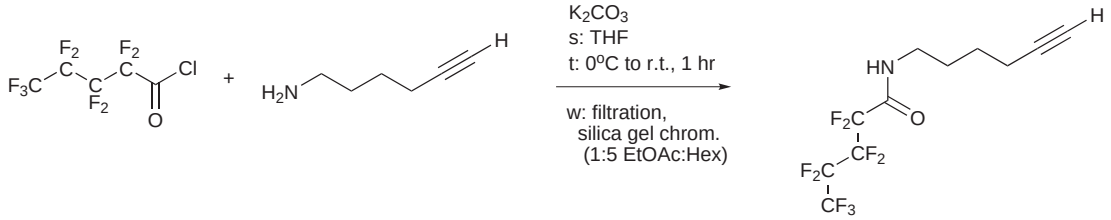


1

General. Hex-5-yn-1-amine, *per*-fluoropentanoyl chloride, ethisterone (**1**) and general chemicals were purchased from Aldrich. *Per*-6'-azido-β-cyclodextrin was prepared as described in Ashton & Stoddart (1996).[1] All solvents were used as supplied without further purification. Distilled water was used in voltage clamp experiments. Analytical thin-layer chromatography (TLC) was performed on E. Merck aluminium-backed silica gel (Silica Gel F254); compounds were identified by charring with a solution of *p*-anisaldehyde in aqueous sulfuric acid and ethanol. NMR spectra were recorded with either (i) a Bruker AMX spectrometer operating at 300 MHz for ^{1}H nuclei, 75 MHz for ^{13}C nuclei, and 282MHz for ^{19}F nuclei, or (ii) a Bruker AMX spectrometer operating at

500MHz for ^{1}H nuclei, and 126 MHz for ^{13}C nuclei. Mass spectra were recorded with a Q-TOF II (MicroMass/Waters, Milford MA) with 4000m/z max quadrapole. Samples were prepared as 1mg/ml solutions in acetonitrile:water, and diluted by a factor of 10. 0.1% trifluoroacetic acid was added to generate more ions.

1.1 Synthesis of 2



2

Scheme 1 Synthesis of **2**.

In a flame-dried 25ml round-bottom flask equipped with a septum and stirbar, anhydrous K_2CO_3 (1.22g, 8.9mmol, 1.5 eqv.) and hex-5-yn-1-amine (579mg, 5.9mmol, 1.0 eqv.) was slurried in dry THF and cooled to 0°C (Scheme 1). *Per*-fluoropentanoyl chloride (2.5g, 8.9mmol, 1.5 eqv.) was added drop-wise to the rapidly stirred solution. The reaction was allowed to warm to room temperature.

After 2 hours, the reaction was quenched by careful addition of MeOH before filtering. The solvent was carefully removed under reduced pressure (the product is slightly volatile). Chromatography on silica gel with 1:5 EtOAc:Hex as eluent gives 273mg product (40%) as a clear, colorless oil.

^{1}**H-NMR** — (300 MHz; $CDCl_3$): δ 6.33 (s, A), 3.37 (q, J = 6.5 Hz, B), 2.19 (td, J = 6.7, 2.7 Hz, C), 1.91 (t, J = 2.7 Hz, D), 1.72-1.62 (m, E), 1.56-1.48 (m, F).

^{19}**F-NMR** — (282 MHz; $CDCl_3$): δ -119.94 (td, J = 12.0, 2.3 Hz, A), -123.42–123.55 (m, B), -125.90 (dtd, J = 12.0, 7.7, 4.2 Hz, C).

^{13}**C-NMR** — (126MHz; $CDCl_3$): δ 157.73 (t, J = 25.7 Hz, A), 83.73 (s, B), 69.33 (s, C), 39.88 (s, D), 28.16 (s, E), 25.52 (s, F), 18.17 (s, G).

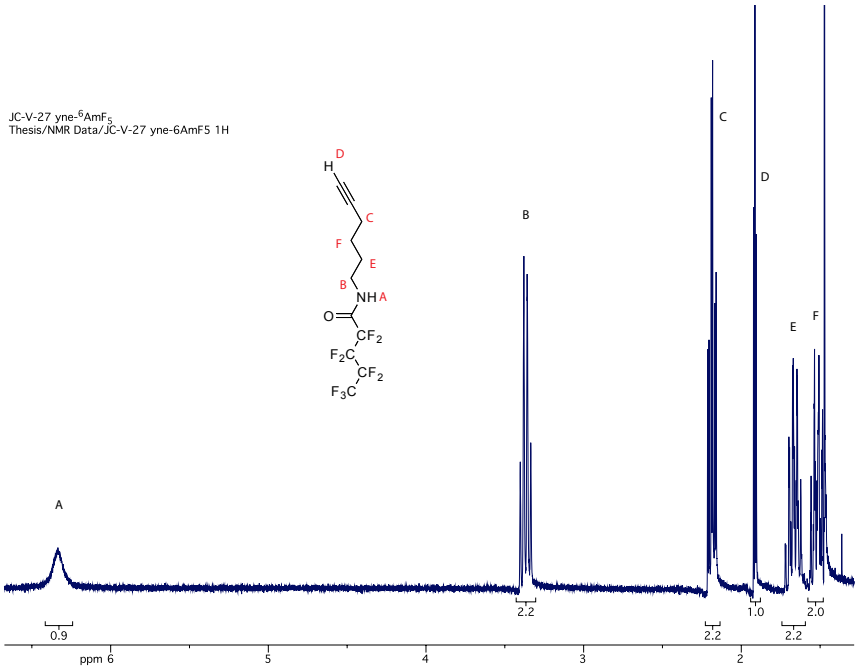


Fig. 1 ^{1}H NMR of **2** in $CDCl_3$

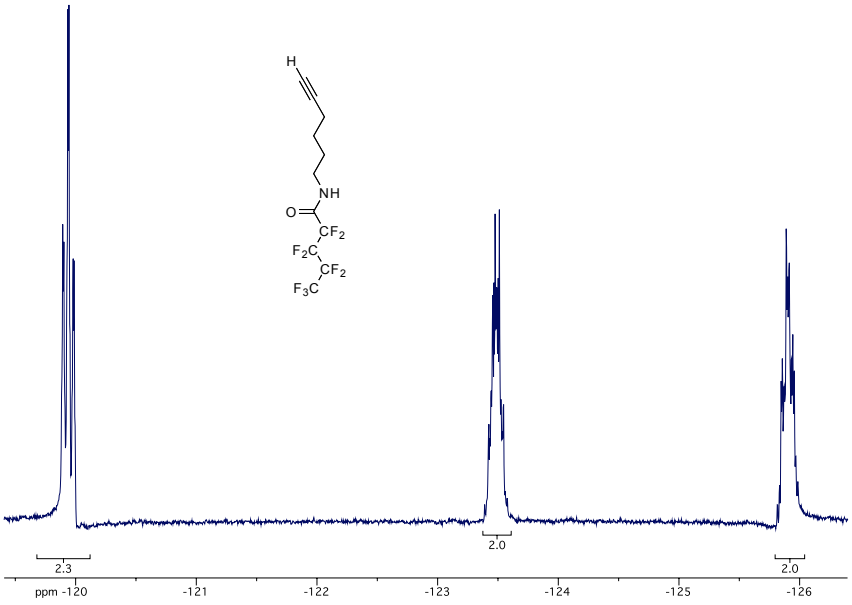


Fig. 2 ^{19}F NMR of **2** in $CDCl_3$

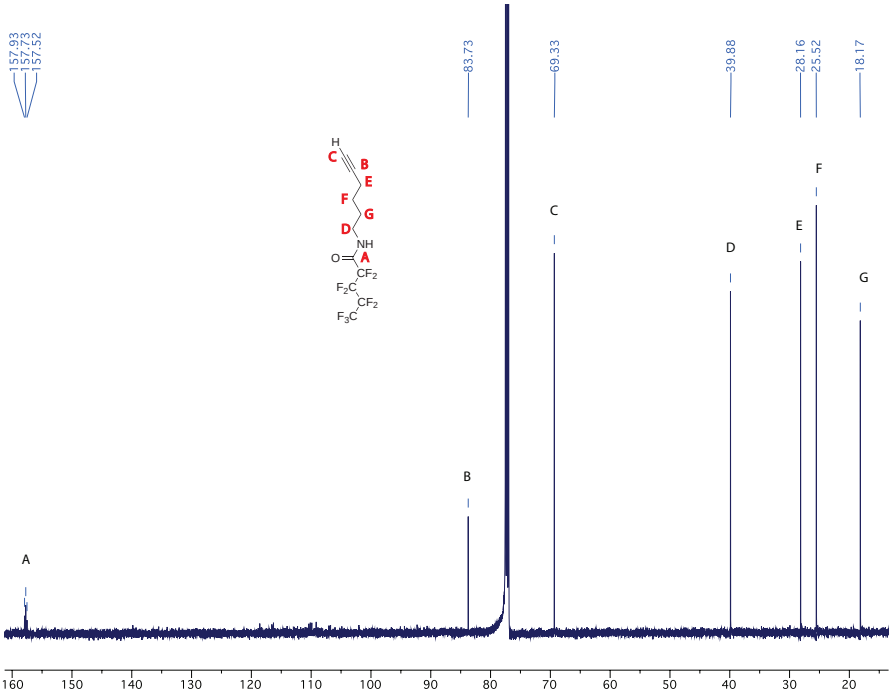
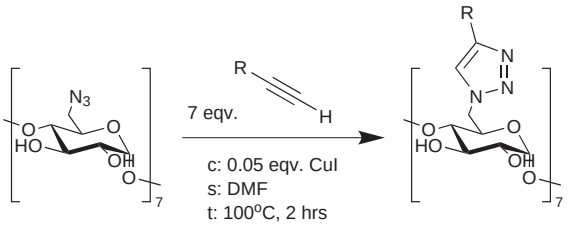
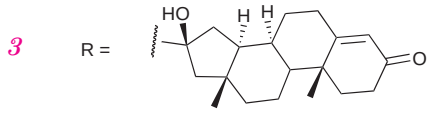
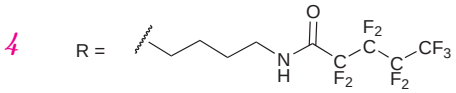


Fig. 3 ^{13}C NMR of **2** in $CDCl_3$

1.2 Synthesis of 3 & 4







In a sample vial equipped with a stir-bar, alkyne (8.0 eqv., 1.15 eqv. per azide), azido-β-cyclodextrin (1.0 eqv.), and CuI (2.1 eqv., 0.3 eqv. per azide) were dissolved in 1:1 DMF:water. The sample vial was capped, sealed with teflon tape, and heated to 100°C. The solution remains heterogenous throughout.

After 2 hours, the dark green-to-orange reaction mixture was cooled to room temperature, and directed applied on a silica gel column, using 10:2:1 acetonitrile:water:$NH_4OH_{(aq)}$ as eluent. The copper salt is retained as a thin blue band on top of the column. Evaporation of solvent with a stream of air[2] gives desired products in 20-75% yields.

1.2.1 Characterization of 3.

Elemental Analysis — Expected C 64.91, H 7.46, N 8.41, O 19.21. Found C 63.43, H 7.30, N 8.29, O 22.98.

Mass Spectrometry (MALDI-TOF) — Expected 3497. Found 1749.5 $[M+2H]^{2+}$

^{1}**H-NMR** — (500 MHz; MeOH-d_4): δ 7.81 (s, V), 5.70 (s, U), 5.15 (d, J = 3.4 Hz, T), 4.77 (d, J = 12.8 Hz, S), 4.60 (s, R), 4.52 (d, J = 15.1 Hz, Q), 4.18-4.16 (m, P), 3.88 (t, J = 9.3 Hz, O), 3.40 (dd, J = 9.8, 3.3 Hz, N), 3.19 (t, J = 9.3 Hz, M), 2.52-2.44 (m, L), 2.26 (m, K), 2.14 (s, J), 2.00 (dd, J = 9.5, 3.3 Hz, I), 1.94-1.88 (m, H), 1.82-1.78 (m, G), 1.67-1.55 (m, F), 1.46-1.36 (m, E), 1.30-1.23 (m, D), 1.03-0.99 (m, C), 0.71-0.65 (m, B), 0.58-0.53 (m, A).

^{13}**C-NMR** — (126 MHz; MeOH-d_4): δ 202.15 (A), 175.07 (B), 154.73 (C), 126.50 (D), 124.35 (E), 103.73 (F), 84.25 (G), 83.00 (H), 74.36 (I), 73.86 (J), 71.75 (K), 55.43 (L), 51.61 (M), 40.17 (N), 38.05 (O), 37.60 (P), 37.14 (Q), 34.94 (R), 34.31 (S), 34.02 (T), 33.35 (U), 25.08 (V), 21.96 (W), 17.86 (X), 15.15 (Y).

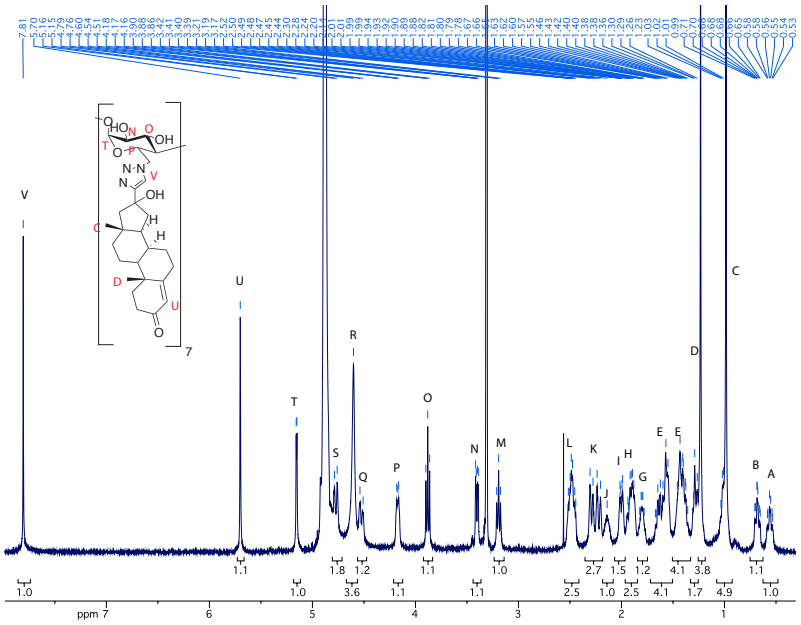


Fig. 4 ^{1}H NMR of **3** in MeOH-d$_4$

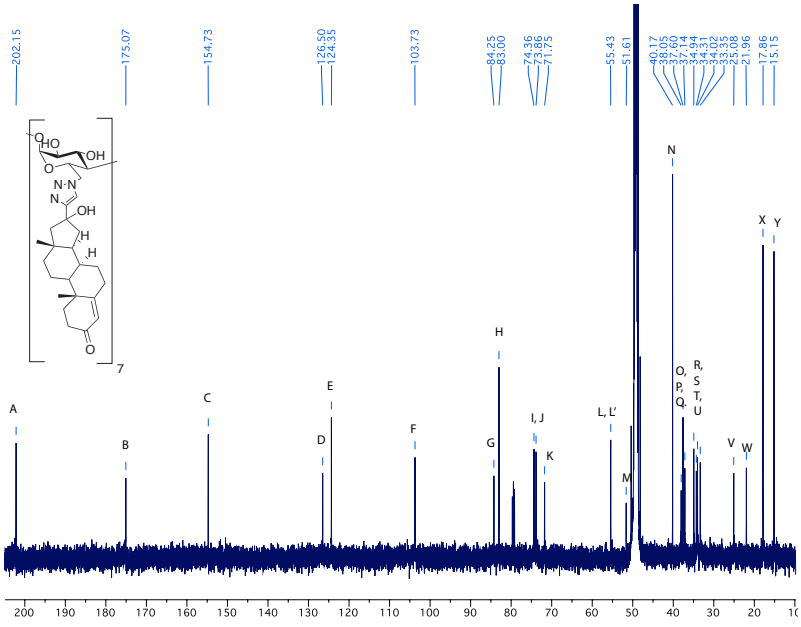


Fig. 5 ^{13}C NMR of **3** in MeOH-d$_4$

1.2.2 Characterization of 4.

Elemental Analysis — Expected C 38.50, H 3.61, N 10.56. Found C 37.59, H 3.73, N 10.40. Analysis for oxygen unavailable due to interference by fluorine.

Mass Spectrometry (MALDI-TOF) — Expected 3710.9. Found 1857 $[M+2H]^{2+}$

^{1}**H-NMR** — (500 MHz; MeOH-d_4): δ 7.68 (s, A), 5.12 (d, J = 3.5 Hz, B), 4.54 (d, J = 12.3 Hz, C), 4.31 (dd, J = 14.4, 6.1 Hz, D), 4.14 (ddd, J = 9.5, 6.1, 3.2 Hz, E), 3.87 (t, J = 9.3 Hz, F), 3.43 (dd, J = 9.8, 3.4 Hz, G), 3.28 (t, J = 9.3 Hz, H), 2.58-2.51 (m, I), 1.59 (d, J = 4.8 Hz, J).

^{13}**C-NMR** — (126 MHz; MeOH-d_4): δ 159.26 (t, J = 25.8 Hz, A), 148.75 (s, B), 125.42 (s, C), 118.88 (qt, J = 287.9, 33.2 Hz, D), 114-106 (m, E, F, G'), 104.00 (s, G), 84.93 (s, H), 74.22 (s, I), 74.04 (s, J), 71.62 (s, K), 51.64 (s, L), 40.87 (s, M), 29.43 (s, N), 27.47 (s, O), 25.73 (s, P).

^{19}**F-NMR** — (282 MHz; MeOH-d_4): δ -120.06–121.01 (m, A), -124.15–124.73 (m, B), -126.93–127.36 (m, C).

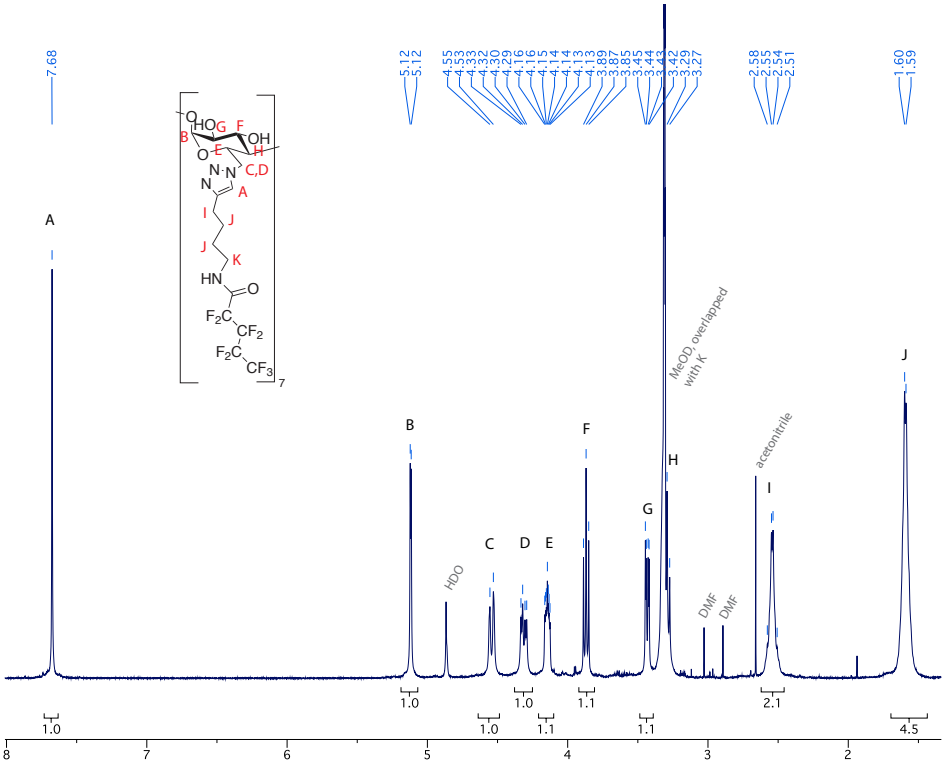


Fig. 6 ^{1}H NMR of **4** in MeOH-d_4

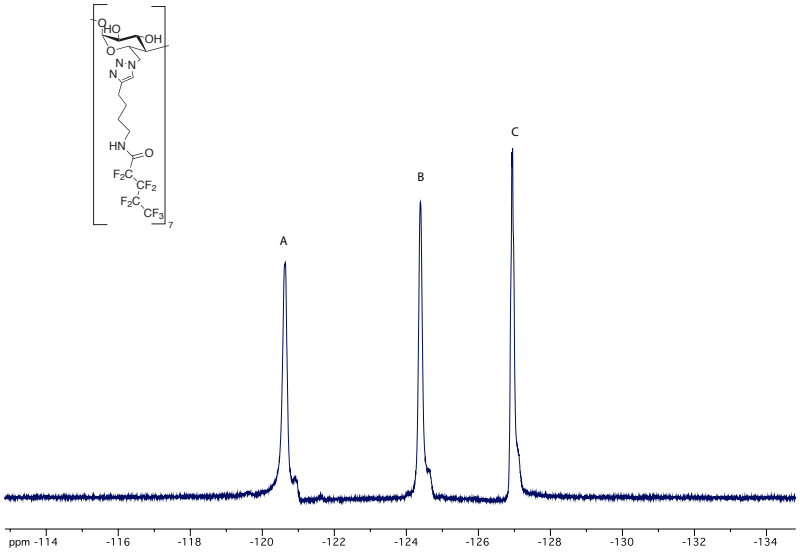


Fig. 7 ^{19}F NMR of **4** in MeOH-d$_4$

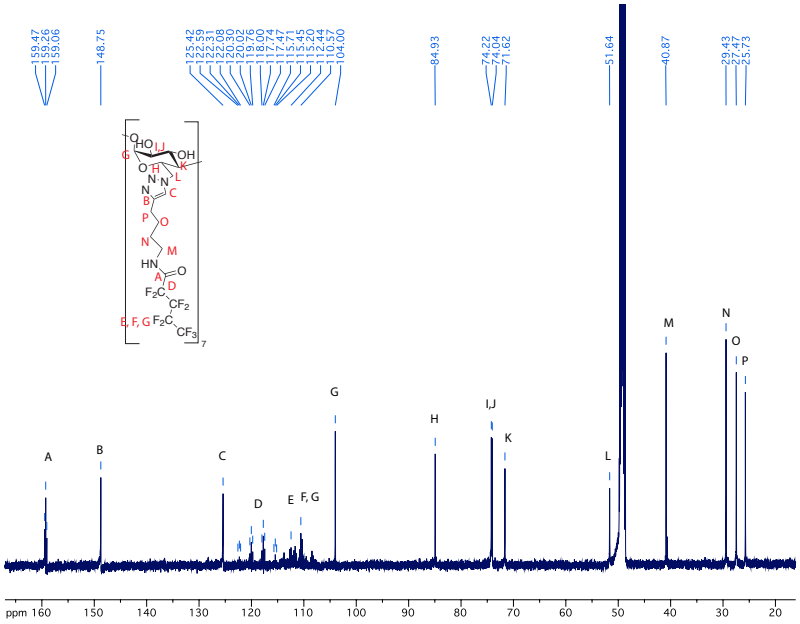


Fig. 8 ^{13}C NMR of **4** in MeOH-d$_4$

2 Voltage Clamp Experiment

A model BC-525A bilayer clamp (Warner Instrument Corp.) was used for planar bilayer experiments. The analogue output was filtered with an 8-pole Bessel filter (Frequency Devices, model 902) and digitized with a 333 kHz digitizer (Axon Instruments, Digidata 1200A). Data acquisition was controlled by the pClamp8 software package (Axon Instruments). Data were collected at 10 kHz, analogue filtered at 1 kHz, and digitally filtered at 50 Hz. The headstage and the bilayer chamber (3 ml polystyrene cuvette with 250µm diameter aperture held in a 5 ml PVC holder) were placed on a floating table and electrically shielded by a grounded aluminum Faraday cage. Agar salt bridges (2 M KNO3 in 1% Agar) were used to stabilize junction potentials and were employed between the electrolyte in each well of the cell and Ag/AgCl electrodes. Electrolyte solutions were prepared from high purity salts and nanopure water.

A stock solution of diphytanoyl phosphatidylcholine (diPhyPC) in chloroform (Avanti Polar Lipids; shipped on dry ice) was divided into sealed glass vials under an argon atmosphere and stored at -12 °C. For use in an experiment, a stream of dry nitrogen was passed through the the vial for 1 h. The dried lipid was diluted with decane to give a solution concentration of 25 mg/ml lipid.

Bilayers were formed by either brushing or dipping: after lipid in decane had been introduced by brushing, a lipid/ decane film formed on the surface of the electrolyte, and bilayers could then be formed by withdrawal of 2-3 ml of electrolyte from the cell holder by syringe to expose one face of the aperture to the air-water interface held in the cell holder, followed by reintroduction of the electrolyte to oppose monolayers across the aperture in the cuvette. Bilayer quality was monitored via the capacitance and stability under applied potential, using the criteria previously described.[3] The measured voltage was applied with respect to the *trans* (cuvette) side of the bilayer, making the *trans* side the relative ground. Digitized data files were analyzed using the pClamp10 suite of programs.

The compounds are introduced to the membrane in two ways, depending on the solvent which the compound was dissolved in.

Direct injection – all injection experiments utilized bilayers that were apparently stable at (100 mV for periods of 20 min or more. Aliquots (1-5 µl) of transporter solutions in MeOH were injected with a microliter syringe as close as possible to the bilayer in the free well of the cuvette holder (*cis* side), and gently stirred with a stream of nitrogen for 5 min.

Pre-mixed into lipid – in this method, 1mol% of compound (in $CDCl_3$ or MeOH-d_4) was added to the diPhyPC/$CHCl_3$ solution, and solvent removed with a stream of N_2, and bilayer membrane prepared by brushing/dipping as described above. Most of the bilayers formed with this method gave bilayers with good quality.

Of the two methods, direct injection is preferable, as it allows monitoring of pristine bilayer prior to compound introduction; this method was used for compound **4**. On the other hand, compound **3** was found to be insoluble in MeOH, and was prepared as a chloroform solution. In this case, premixing is necessary.[4]

The apparently erratic behaviour typically appears within 20 min of compound introduction, and persists over period of hours. Once stabilized, continuous data acquisition of at least 30-60 min is required to provide sufficient statistical power for the power-law analysis.

3 Power Law Fitting Procedure

Fitting experimental data to a power law requires two distinct steps. The first step transforms the irregular current trace into a list of opening times; this list is then fitted to a power law distribution.

3.1 Event List Generation

Manipulation of the digitally filtered traces was carried out using Clampfit 10 of the pClamp suite. A customized threshold search was used to generate the list of events. Detail procedures follows:

1. Open and apply appropriate filter to acquired data trace.
2. Initiate a template search (Fig. 9)

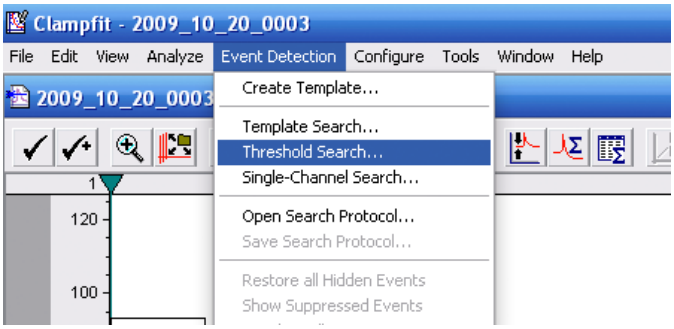


Fig. 9 Template search

This opens a new pane to allow adjustment of search parameters (Fig. 10). The significance and choices of parameters are indicated below:

Threshold defines the boundary for which the crossing and subsequent re-crossing constitute one event. This was set across the fluctuating section of the trace to maximize the number of events. Within that segment, α is insensitive to the choice of threshold.

Baseline defines the current flux for the event. This is not used in the analysis, and is generally set to zero for convenience.

Minimum duration was fixed at 50ms.

Pretrigger and Post-trigger length Clampfit allow optional splicing of individual events. These parameters configures the bracketing of spliced events. This is not used in our current analysis.

3. Select all events that meet criteria (button A in Fig. 11).

   This should result in a list of event selected highlighted in blue (Fig. 12). Quit event detection to generate the spreadsheet of events (button B).

4. The results of the threshold search is automatically logged in a *Results* spreadsheet within Clampfit (Fig. 13). Duration (the difference between *event start* and *event end*, columns

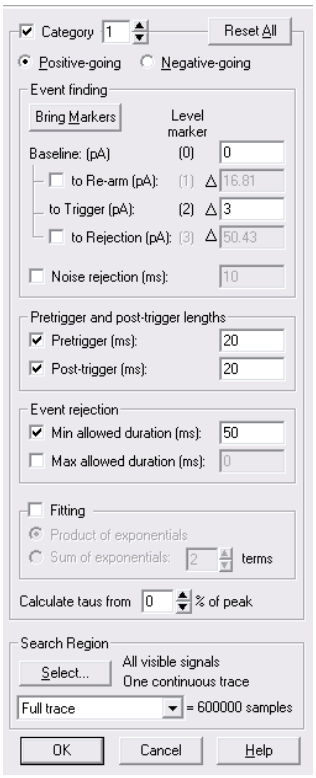


Fig. 10 Search parameters adjustments.

Fig. 11 Select all events.

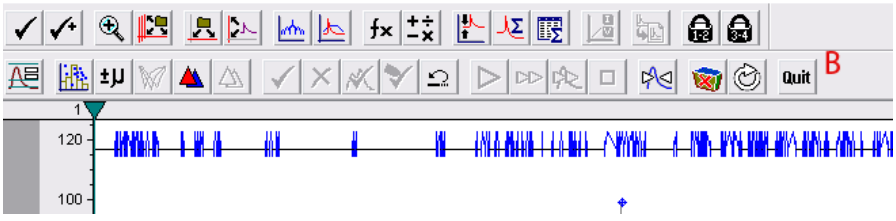
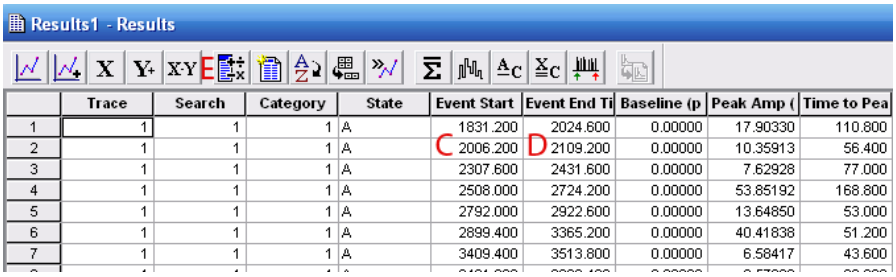


Fig. 12 After event selection.

C and D) can be calculated using "column arithmetic" function (button E). The resulting list can then be extracted into a plain text file for power law fitting.



	Trace	Search	Category	State	Event Start	Event End Ti	Baseline (p	Peak Amp (	Time to Pea
1	1	1	1	A	1831.200	2024.600	0.00000	17.90330	110.800
2	1	1	1	A	2006.200	2109.200	0.00000	10.35913	56.400
3	1	1	1	A	2307.600	2431.600	0.00000	7.62928	77.000
4	1	1	1	A	2508.000	2724.200	0.00000	53.85192	168.800
5	1	1	1	A	2792.000	2922.600	0.00000	13.64850	53.000
6	1	1	1	A	2899.400	3365.200	0.00000	40.41838	51.200
7	1	1	1	A	3409.400	3513.800	0.00000	6.58417	43.600

Fig. 13 Event Information

3.2 Power Law Fitting

The list of opening durations, obtained above as a plain-text file, can then be fitted using Clauset *et al.*'s method, implemented in python by Adam Ginsburg[5]. The code performs the Maximum Likelihood Estimate fit, and provides α, x_{min}, n, and p-value as outputs. We used the Enthought distribution of Python (6.1.1, python 2.6.4).[6] This cross-platform distribution includes all the required libraries (NumPy, SciPy, and Matplotlib) in one convenient binary.

References

[1] P. R. Ashton, R. Koniger, J. F. Stoddart, D. Alker and V. D. Harding, *The Journal of Organic Chemistry*, 1996, **61**, 903–908.

[2] This is preferable to the usual procedure of rotary evaporation; some amphiphilic products foam under vacuum.

[3] T. M. Fyles, R. Knoy, K. Mullen and M. Sieffert, *Langmuir*, 2001, **17**, 6669–6674.

[4] With chloroform as the solvent, droplets of the immiscible solvent partition to the bottom of the electrolyte during the direct injection technique.

[5] http://code.google.com/p/agpy/wiki/PowerLaw.

[6] http://www.enthought.com/.