Supporting information for: *Cyclodextrin Ion Channels*

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**Voltage Clamp Data Acquisition**

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 330 kHz digitizer (Axon Instruments, Digidata 1200A). Data acquisition was controlled by the pClamp8 software package (Axon Instruments). Data were collected at 10 Hz, analogue filtered at 1 Hz, and digitally filtered at 50 Hz. The headstage and the bilayer chamber (3 mL polystyrene cuvette with 250 µm 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 vial for 1 hour. The dried lipid was diluted with decane to give a solution concentration of 25 mg/mL in 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\(^1\). 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 in which the compound can be dissolved:

*Direct injection* - all injection experiments utilized bilayers that were apparently stable at 100 mV for periods of 20 minutes 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 minutes.

*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. Following direct injection, channel behaviour typically appears within 20
minutes of compound introduction, and persists over period of hours. Once stabilized, continuous data acquisition of at least 30-60 minutes is required to provide sufficient statistical power for the power-law analysis; shorter acquisition periods (10 minutes) are sufficient to characterize other types of behaviors.

**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.

**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. The threshold was set across the fluctuating section of the trace to maximize the number of events. Within that segment, \( \alpha \) is insensitive to the choice of threshold. A minimum duration was fixed at 50ms. The threshold search automatically logs event start and event end from which the duration can be calculated. The resultant values were exported to the fitting program.

**Power Law Fitting** The list of opening durations, obtained above as a plain-text file, can then be fitted using the method of Clauset et al\(^2\), implemented in python\(^3\). The code performs the Maximum Likelihood Estimate fit, and provides \( \alpha \), \( x_{\text{min}} \), \( n \), and p-value as outputs.

**Summary of bilayer activity**

Annotated activity grids, as well as full conductance records (and expansions where appropriate), are provided below for every compound studied. The activity grids were prepared as previously described\(^4\). The summaries are arranged first by compound, then individual experiments. Within each experiment, the first page(s) summarizes the experimental conditions as well as activity grids charted; subsequent pages shows the full conductance record as the top panel, with expansions indicated by corresponding letters.


bad bilayer - no compound added
Broken bilayer
opening continued in 0001
> 400 sec

36pS
same opening continued from 0000

36ps

A

B

A

B
very large noise
JC-133 JC-W-2 bCD-6AmF5 1M CsCl pH 7

- Lipid
- Electrolyte 2.7/4.2uM
- Contact injection
- Brush transfer
- Adamantyl guest

Baseline drift, large leakage
large leakage
JC-135 JC-W-2 bCD-6AmF5 1percent 1M CsCl pH 7

- Electrolyte 5.8/3.8 mM
- Lipid 1%
- Contact injection
- Brush transfer

Adamantyl NH₂ addition made halfway through exp.

Potential/mV

Time/s

Large leakage

AdNH₂ addition made halfway through exp.
fractal?
JC-150 bCD-6AmF5 1percent 1M CsCl pH 4.5

Potential/mV

0 100 200

-100

-200

Time/s

4000 6000 8000 10000 12000

l lipid 1%

electrolyte

contact injection

brush transfer

Adamantyl guest

lipid

electrolyte 1.4/0.9uM

AdNH2 addition made halfway through exp. 0006

AdNH2 addition made halfway through exp. 0006

only 4 sec trace
There may be structure under there, but it’s not obvious where to start/end.
[JC-72] bCD-6-Es-8 1M CsCl pH 7 survey

- Electrolyte
- Lipid
- Contact injection 14.6uM (insol)
- Brush transfer

Inexplicable noise or genuine small, frequent activity?

Structure instable to potential changes

Fractal?
structure instable to potential changes
Is there structure here?

fractal?
electrolyte 12.5/8uM
lipid 1%
contact injection
brush transfer
electrolyte
lipid
Adamantyl guest

mixed with fractal-like section
potential changes
Also an open state (see dip) not charted here
all dips ~200-300pS

290 pS
0.4s
JC-149 bCD-6Es8 1percent 1M CsCl pH 4.5

from lab book description -
no trace acquired
[JC-72] bCD-6-Es-8 1M CsCl pH 7 survey

Potential/mV

0  100  200
-100
-200

Time/s

0  2000  4000  6000  8000  10000

- lipid
- electrolyte
- contact injection 14.6uM (insol)
- brush transfer

- structure unstable to potential changes

- inexplicable noise or genuine small, frequent activity?

- fractal?
structure instable to potential changes
Is there structure here?

fractal?
potential changes
Also an open state (see dip) not charted here
all dips ~200-300pS

290 pS

0.4s
From lab book description - no trace acquired
potential changes

A
[JC-77] bCD6Am8 1M CsCl pH 7 homoAdd

Potential/mV

Time/s

l lipid
electrolyte 6.4/9.6uM
contact injection
brush transfer

broken bilayer
[JC-73] bCD6Ur8 1M CsCl pH 7 survey run 1 perc

0 2000 4000 6000 8000 10000 12000 14000
0 100 200

Potential/mV

Time/s

lipid 1%
electrolyte
contact injection
brush transfer

continuation of 0007
potential change
This is a very good illustration of flicker/inverse flicker
This is a very good illustration of flicker/inverse flicker Continuation from 0007.
electrolyte
lipid 1%
contact injection
brush transfer

Broken bilayer

Nothing seen in remainder of trace
It is not clear what I saw in this experiment
electrolyte
lipid 1%
contact injection
brush transfer
electrolyte 125mM
lipid
Bu4NBr
artifacts?
NOT ASSIGNED

noisy looking,
I feel this is too regular to be fractal
I think these are artifacts.