tethered
membrane
technology

tethaPod™

eDAQ

www.edaq.com

Overview

The tethaPod™, Figure 1, is designed to work with tethaPlate™ inserts containing tethered phospholipid bilayer membranes. An automated, highly optimised, multifrequency impedance methodology is used to accurately determine the transmembrane conductivity (resistance) of the bilayer. The addition of ion channel proteins to the membrane increases the conductivity, while the subsequent addition of channel blockers or activators can further modify the conductivity.

The use of a six–chambered tethaPlate means that sufficient replicates can be done in the one experiment to generate statistically valid results, making the system ideal for dose–response studies.

Tethered Membranes

Within each chamber of the tethaPlate a phospholipid bilayer membrane is held in place above a planar gold electrode by a series of molecular 'tethers', Figure 2. These tethers comprise a disulphide anchor to the gold surface, a central hydrophilic PEG (polyethylene glycol) molecular chain, and finally a lipohilic phytanyl chain which embeds in the lipid bilayer. The tethers are separated on the gold surface by a set of shorter 'spacer' molecules which comprise only the disulphide anchor with hydrophilic PEG chain. The ratio of tethers to spacers is set at 1:10, which has been found to support a phospholipid membrane that accommodates membrane proteins of up to 40000 Da, Figure 3. These proteins can then form dimers, pentamers, etc., as required, to create the final form of the ion channel. Small ion channels such as gramicidin (1882 Da) and valinomycin (1111 Da) are equally well accommodated.

Different tether/spacer ratios can be supplied on custom orders but ratios of less than 1:100 are not recommended as the stability of the phospholipid bilayer is compromised.

Membrane Proteins

Ion channels are protein molecules, or assemblages of such molecules, that are housed in, or across, a phospholipid membrane. Simple ion channel polypeptides (such as gramicidin, valinomycin, magainin) are available commercially in pure form. Larger proteins may also be commercially available but are often supplied in a detergent or lipid matrix. However most such larger proteins are made either in–house or by a contract biotechnology company using bacterial culture/genomic techniques.

Figure 1
tethaPod™

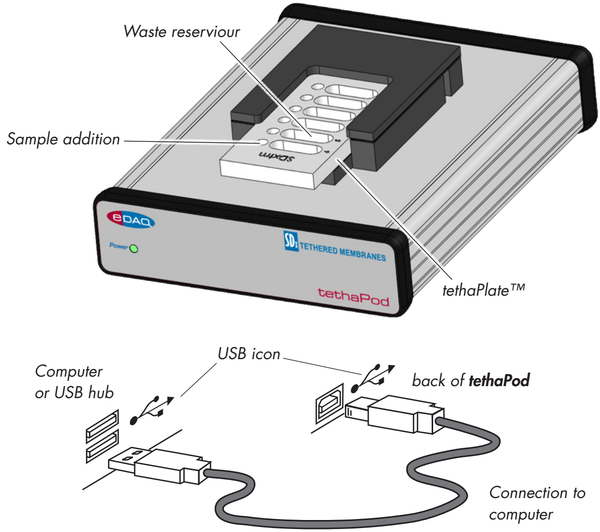


Figure 2
Cross section of tetherPlate chamber showing tethered membrane on gold electrode surface

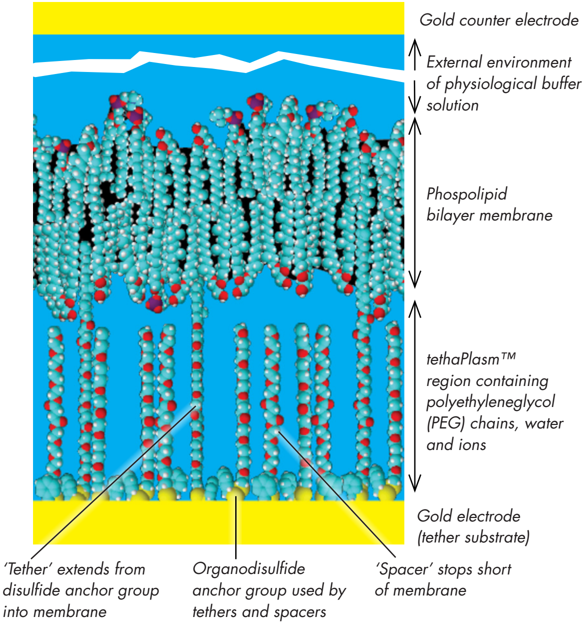
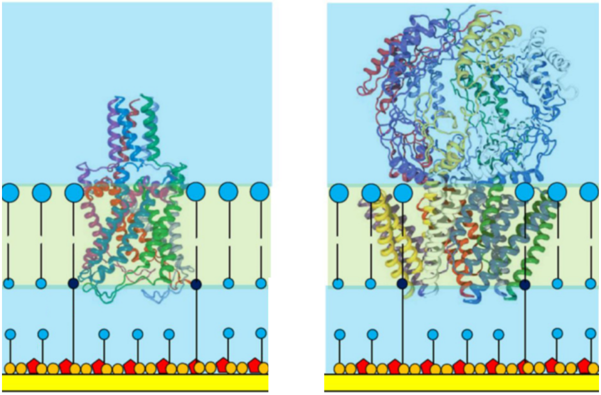


Figure 3
Ion channel proteins sitting in tethered membranes (roughly to scale)



Serial Communication

The tethaPod unit sends data to the computer over a USB interface using a serial (RS232) protocol. Normally use the supplied tethaPod software to monitor the signals. Terminal emulation software (such as Hyperterminal, or Tera Term) can also be used, if diagnosis is required for anomalous readings.

For best results when recording signals:

- *set your computer to 'never' go to sleep.*
- *turn 'Windows updates' off if you are using the software overnight.*
- *use the latest version of the tethaPod software.*

Installation

Install the software supplied on the USB memory stick with your unit. This will install the software and USB drivers for the tethaPod.

Connection

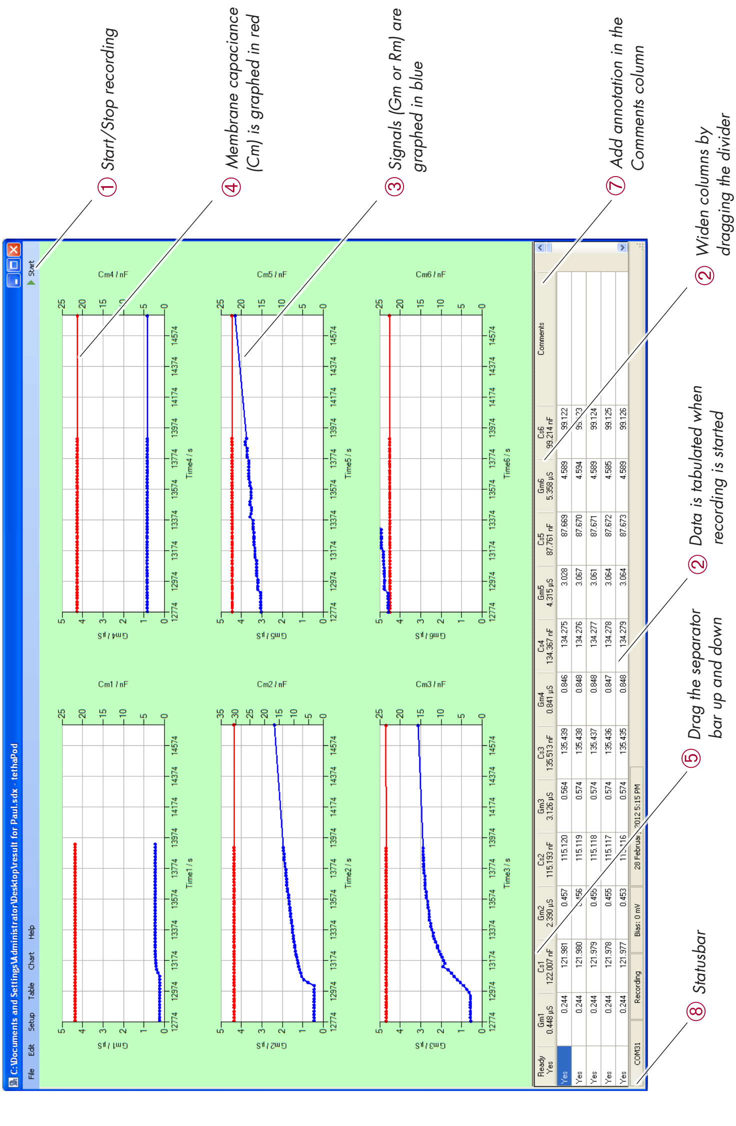
Connect the tethaPod unit to the USB port of a computer or, USB hub, Figure 1. USB ports are usually denoted with a trident–like, ⭠ , icon.

tethaPlate

The tethaplate can be inserted into the socket on the top of the tethaPod unit by gently pushing it in. Remove by gently pulling the tethaPlate towards the front of the unit.

Figure 4

Software features



tethaPod Software

The tethaPod software enables Windows XP, or later, computers to monitor signals from the six chambers in the tethaPlate inserts using the tethaPod hardware. Data can be copied and pasted to other software for further analysis or reporting, or saved in text format which can be read by spreadsheet or word processor software.

The software can also be used to open old data files from previous experiments. The names of tethaPod data files are assigned the extension '.sdx'.

Software Features

Figure 3 shows some of the basic features available when you use a tethaPod software.

① To **start/stop** collecting data click the Start/Stop button. (The button toggles between the two modes.)

② When started, the software will **log data** to a table of parameters (see below for a description of the various parameters), and

③ graph membrane conductance (or resistance, see Chart menu, below) versus time.

④ Membrane capacitance (red traces, see Chart menu) can also be graphed.

⑤ The relative proportions of screen space devoted to the graphs and the tabulated data can be altered by dragging the separator bar.

⑥ The columns of tabulated data can be widened.

⑦ User annotations (for example 'sample addition' can be added in the Comments column.

⑧ A status bar keeps track of file creation time and date, and hardware activity.

Parameters

The most important parameter to be determined is R_m (membrane resistance, in kohm), or G_m (membrane conductance, in μS). These two quantities are related, $G_m = 1000/R_m$. For an intact membrane, without ion channels, R_m is usually in the range 1000 – 50000 kohm,

Note that G_m is directly proportional to the transmembrane current (the number of ions per second passing through the membrane) and is often the parameter of choice for dose/response and similar pharmacological studies.

The membrane conductance, G_m is typically less than 1 μS for an intact phospholipid membrane, but can increase dramatically when an active ion channel protein is introduced, becoming as high as 1000 μS.

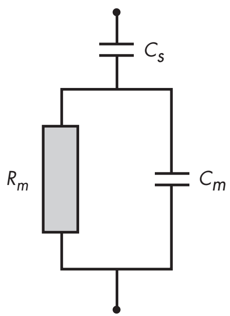
The membrane capacitance, C_m, should remain fairly constant (usually about 10 – 20 nF) during most experiments. Any large change that occurs indicates a significant disruption of the membrane, or its edge seals, and the values obtained for R_m and G_m are invalidated.

The series capacitance, C_s, is a property of the gold electrode itself and not related to the membrane per se. It should remain at a constant value of about 100 nF. Any large change occurring during the experiment indicates a severe problem with the sample, and the values obtained for R_m and G_m are invalidated.

The *GoF* parameter is quantity that defines the 'goodness of fit' to the data by a model of the membrane as a theoretical arrangement of a pair of capacitors and an electrical resistor, and a *GoF* of zero would be a perfect fit. A *GoF* value of 0.1 (or less) indicates excellent agreement. A threshold constituting an acceptable *GoF* can be set — see the **Set GoF Threshold** command in the **Table menu**, below. Data points with an unsatisfactory (large) *GoF* can be marked in green on the various graphs, or omitted altogether.

The *State* parameter can have values of 1 to 4 and indicates the progress of a measurement cycle in each chamber of the tethaPlate. A data point is not finalised until State 4 is achieved.

Figure 5
Tethered membrane parameters

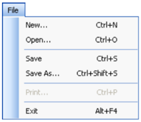


Equivalent circuit used to analyse tethaPod signals

Parameter	Value
R_m	>1000 kohm
G_m	<1 μS
C_m	10 – 20 nF
C_s	100 nF

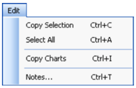
Typical values for a phospholipid bilayer. R_m decreases (and G_m increases) when an ion channel is active.

Menus and Other Features



File menu

The **File** menu has standard commands for opening new or existing Pod-Vu files, saving files, printing the data, and exiting the software. The **Save As** command can be used to save data as text files for exporting to other software.

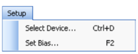


Edit menu

Use the **Copy Selection** command in the Edit menu to transfer selected data in the table by pasting into spreadsheet and word processor documents. Use the **Select All** command to select all the data in the table

The **Copy Charts** command enables pasting of the signal graphs into other documents.

Use the **Notes** command to make comments and observations about the experiment — these annotations are saved in the data file.



Setup menu

In most cases **Set Bias** to zero. A non-zero bias voltage creates an electric field, required by some ion channel proteins to align themselves across the phospholipid membrane. The bias can be adjusted between ±500 mV before starting recording. The bias value is reported in the **Status Bar**.

If multiple tethaPod units are connected to the same computer it may be necessary to identify which unit is being used by using the **Select Device** command.

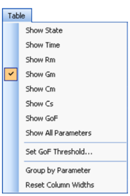


Table menu

This menu defines what parameters are to be displayed in the table area. Whatever selection is made, all parameters are always stored in the file and can be re-displayed or exported at a later stage. Data columns can be grouped by tethaPlate chamber number, or by similar parameter type (use the **Group by Parameter** command).

Set GoF Threshold defines a value above which *GoF* is considered unsatisfactory.

Column widths can be changes by dragging the column separators in the column titles row, Figure 4 ⑥. The widths of the columns can be returned to their default values using the **Reset Column Widths** command.

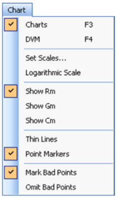


Chart menu

This menu controls how the G_m, R_m and C_m signals are to be graphed. Choose the **Charts** command to graph the signals, Figure 4 ③, ④. The **DVM** (digital voltmeter) command replaces the graphs with large format numerical displays. If both the **Charts** and **DVM** commands are unchecked then neither will be displayed and the tabulated results will fill the window.

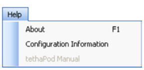
Note that you can show the graphs of G_m versus time, or R_m versus time, but not both simultaneously. These graphs are shown in blue. The graph of C_m versus time is shown in red.

The **Set Scales** command accesses controls to specify the Y axis limits for the various graphs.

The **Logarithmic Scale** command uses a logarithmic Y axis for graphing the G_m, or R_m signals.

These signals are shown as lines joining the data points, optionally with **Point Markers** and/or with **Thin Lines**.

Data points with a large (unsatisfactory) *GoF* value (see Table Menu, above, to define the threshold) can be omitted from the graphs (**Omit Bad Points**), or designated with a green colored marker (**Mark Bad Points**).



Help menu

The **About** command displays the tethaPod software version number, and computer configuration information.

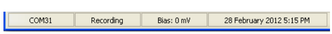
The **Configuration Information** command gathers information about your computer system that can then be email to us for technical support.

Use the **tethaPod Manual** command to access a pdf file of this manual.

Start/Stop button

The **Start**/Stop command starts and halts data collection, Figure 4 ①.

Status bar



Status bar

The Status bar, Figure 4 ⑧, shows the virtual COM port that the tethaPod is using, whether or not the software is collecting data, bias voltage, and the date and time at which data collection began.

Specifications

tethaPod

Channels:	6
Communication:	USB virtual serial port (VSP)
VSP driver:	http://www.ftdichip.com/Drivers/VCP.htm
COM port settings:	9600 baud; 8 bits; 1 stopbit; no parity; flow NONE
AC frequencies:	0.125, 0.25, 0.5, 1.25, 2.5, 5.0, 12.5, 25, 50, 1000 Hz
AC amplitude:	±10 mV (20 mV p-p)
DC bias voltage:	±0.5 V maximum
Membrane resistance:	10 – 5000 kohm (0.2 – 100 μS)
Dimensions (h x w x d):	50 x 129 x 168 mm
Weight:	~470 g
Operating conditions:	0 — 40 °C, 0 — 90% humidity (non condensing)

tethaPlate

Cartridge body:	white polycarbonate, 75 x 39 x 7 mm
Chambers:	6
Gold electrode substrate:	transparent polycarbonate slide, 75 x 25 x 1 mm
Tethered electrode dimensions:	3.0 x 0.7 mm (area 0.21 mm^2)
Flow channel depth:	0.15 mm
Loading port:	4.5 mm diameter circle, 4.5 mm deep
Waste port:	22 x 7.5 mm, 4.5 mm deep
Tethers:	benzyl-disulphide-bis-tetraethyleneglycolmonophytane
Spacers:	benzyl-disulphide-bis-tetraethyleneglycol
Weight:	~11 g

Phospholipid Mix

Volume:	1 mL
Total lipid concentration:	3 mM in ethanol
Lipid mixture:	diphytanyletherphosphatidylcholine 70% and glycerodiphytanylether 30%

Inserting Ion Channels

To add solutions to a tethaPlate simply pipette a small volume to the input port of a chamber (Figure 1). The solution will then pass through the chamber, and across the tethered membrane coated electrode. Excess solution will appear at the waste port and can be removed by pipette if desired. Take care to avoid the introduction of air bubbles into the chamber.

There are several common protocols used for incorporation of ion channels into a tethered membrane:

1. Spontaneous insertion into a preformed tethered membrane. This is has been used for CLIC (the protein chloride intracellular ion channel), or the bacterial ion channel alpha–hemolysin. The ion channel is added in a solution of PBS (physiological buffer solution). Cholesterol and/or the presence of charged lipids (such as phosphatidylethanolamine) can sometimes accelerate incorporation, as can lower pH (down to pH 4.5 or so).

   A variant of this approach is to use detergent (for example Tween20®) assisted insertion which has been used for the mechanosensitive channel MscL. After insertion fresh PBS is used to rinse the chamber, removing the excess ion channel and other reagents. It is also possible for fusion of an ion channel–containing proteoliposome to take place with a preformed tethered membrane

2. The second major approach is to form the tethered membrane in the presence of the ion channel (with or without a detergent). For example a liposomal suspension of voltage dependent anion channel (VDAC) can be added to the chamber of a tethaPlate so that the lipids comprising the liposomes form a bilayer while the ion channel is simultaneously incorporated into the membrane. Bacterial sodium channel (NaChBac), incorporates at time of membrane formation with Cymal-5 detergent. Small conductance mechanosensitive channel (MscL) requires charged lipids (such as phosphatidylethanolamine) to accelerate insertion and can be incorporated at time of membrane formation from DDM detergent.

Once inserted, by whatever means, protein ion channels usually exhibit good stability in tethered membranes, and can often be used for long periods (several days or more) provided that the tethaPlate is stored below 4°C when not in use.

TradeMarks

tethaPod, **tethaPlate** and **tethaPlasm** are trademarks of SDx Tethered Membranes Pty Ltd and used by eDAQ under license.

Warranty

eDAQ Pty Ltd warrants **tethaPod** systems, to be free of defects in material and workmanship for one year from the date of purchase. eDAQ Pty Ltd will repair or replace defective equipment as appropriate.

To obtain a warranty repair/replacement you must first notify us before return of the instrument and we will issue you with a RAN (return authorization number). You must ship the defective product at your expense. We will pay return shipping. The product should be packed safely (preferably in its original packaging) and have the RAN on the shipping label. Returns sent without a RAN may be refused delivery.

This warranty does not cover hardware that has:

- been modified by the user in any way;
- been subjected to unusual physical, electrical, or environmental stress. This includes damage due to faulty power sockets, inadequate earthing, or power spikes or surges;
- been damaged because of incorrect wiring to ancillary equipment, or because of substandard, connectors or cables; or
- had the original identification marks removed or altered.

Software License

You have the non-exclusive right to use the supplied **tethaPod** software. (Your employees or students, for example, are entitled to use it, provided they adhere to this agreement.) Each separate purchase of the **tethaPod** software licenses it to be used on two computers at any given time (on one computer for data acquisition with **tethaPod** hardware units, and on a second computer for the review and analysis of existing data files). Although multiple copies of a program may exist on several computers, more than two copies must not be used simultaneously. Departmental/company licences are available if you wish to run more than two copies simultaneously.

Jurisdiction

eDAQ Pty Ltd is bound by the laws of New South Wales in Australia, and any proceedings shall be heard by the Supreme Court of New South Wales in Australia.

Technical Support

Technical assistance is available via email. Ensure that you are using the latest version of the **tethaPod** software, which can be downloaded at http://www.edaq.com/support.php Please describe the problem with as much detail as possible. Include an example data file, if appropriate. Please also state:

- the serial numbers of your **tethaPod** units; and
- information gathered with the Configuration Information command in the Help menu.

We endeavor to answer all your questions, but in some cases, for example where the problem relates to the other equipment that you are using, a nominal fee may be charged.

Disclaimer

1. eDAQ Pty Ltd reserves the right to alter specifications of hardware and software without notice.

2. No liability can be accepted for consequential damages resulting from use of eDAQ products.

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