

Membrane Conduction and Ion Channels:

Key Learning Objectives:

1. Bilayer lipid membranes (BLM) are the major constituent of cell membranes.
2. BLMs block the passage of ions such as Na^+ , K^+ , Cl^- and Ca^{++} .
3. Ion channels penetrate cell membranes permitting the passage of ions across the membrane.
4. Ion carriers don't penetrate through membranes but shuttle ions across membranes.
5. The bacterial protein Hemolysin is an example of an ion channel.
6. The bacterial polypeptide Gramicidin is an example of an ion channel.
7. The bacterial polypeptide Valinomycin is an example of an ion carrier.
8. Hemolysin is not selective and transports mono and divalent anions and cations.
9. Gramicidin transports monovalent cations such as Na^+ & K^+ across BLMs but does not transport anions such as Cl^- or multivalent ions such as Ca^{++} .
10. The ion carrier Valinomycin selectively transports only K^+ ions across a BLM with a selectivity of better than 10,000:1, $\text{K}^+ : \text{Na}^+$.
11. Ion channels are far more efficient ion transporters than ion carriers.
12. Ion channels and carriers are far poorer conductors of ionic electrical charge than a copper wire is a conductor of electronic electrical charge;
13. A convenient tool for studying the ion transport properties of BLMs is a tethered membrane.
14. Tethered membranes are more stable and easier to study than cell membrane because they are chemically attached to a solid metal (gold) surface.
15. This practical demonstrates the formation of a tethered membrane and the inclusion in it of an ion channel or an ion carrier.
16. Membrane thickness and ion channel transport rates are measured as part of this practical class.
17. The student is introduced to tethered membranes as platforms for constructing nanoscale devices.
18. The student performs the fabrication of a tethered membrane, inserts hemolysin, gramicidin and Valinomycin, and measures the conductance ad selectivity of each type of ionophore.
19. The student answers a series of simple questions based on the practical exercise to show they understand these learning objectives.

Background:

Cell Membranes

Cell membranes are essentially the walls of water-filled soap bubbles. They are self-assembled supramolecular structures formed by amphiphiles, or compounds that have polar segments that strongly attract water and non-polar segments that do not. This results in the non-polar segments being excluded from the aqueous phase and assembling into bimolecular sheets which eventually form closed spheres. This is believed to be the driving force for the evolution of cell membranes.

The energies involved in this process are:

- a positive entropy contribution, due to water molecules in the bulk being more disordered than water at a hydrophobic surface. This is because water at a hydrophobic surface will be oriented away from the surface as the water molecules hydrogen bond to each other and not to the surface. This preferred orientation results in more order and thus less entropy.
- a positive enthalpy contribution due to the excluded segments being attracted to each other.

The amphiphiles we are interested in are known as lipids and thus the cell-like structures they form when dispersed in water are known as liposomes. Liposomes can 10 nm to hundreds of micrometres in diameter but all have walls that are approximately 4 nm thick, and are nearly impermeable to ions such as Na^+ , K^+ and Cl^- . The 4 nm thick lipid bilayer membrane that forms the wall of a liposome is similar to that found in cell membranes, whether they are from bacteria, plants or animals, including humans.

Alterations in membrane ionic permeability is the basis of:

- signalling between neurones in the brain, and between neurones in the sympathetic and autonomic nervous systems.
- the senses of sight, sound, taste, touch and smell in animals, and related functions in plants and bacteria.
- mitochondrial metabolism and bioenergetics.

Cell membrane biochemistry is very important in medical research and a core interest of the Pharmaceutical Industry when searching for drugs to address a wide range of medical conditions. Compounds that interact with membranes are also important in understanding the effects of many venoms, toxins, and some chemical warfare agents.

Tethered membranes:

Tethered membranes provide a planar phospholipid bilayer over a relatively large surface area (2 mm^2) that is a convenient way to study of ion transport. Otherwise it is necessary to use very small liposomes or cells with cumbersome microelectrode techniques. The tethering of the membrane is achieved using sulphur chemistry to gold (gold is not totally unreactive and possesses a chemistry with sulphur). Molecular tethers are thus molecules that possess a sulphur group, polar linkers and a hydrophobic segment that embeds in the lipid bilayer. The polar linkers allow the existence of an aqueous layer, between the gold electrode and the membrane. Spacer molecules (sulphur group and polar linker only) are positioned between the tethers. Figure 1 shows a typical assembly process.

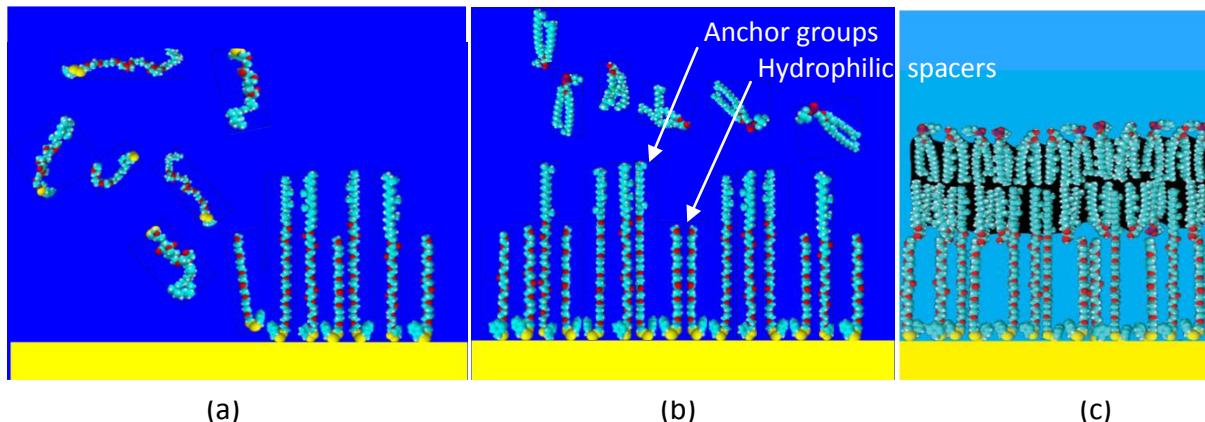


Figure 1.

(a) Ethanol solutions (■) containing 0.4mM disulphides are exposed to pure fresh gold for 30minutes. The molecules collide with the gold and sulphur-gold bonds form, causing the self assembly of a lipid-spacer monolayer. Ten percent of the molecules are hydrophobic lipidic anchor groups, and ninety percent are hydrophilic spacers. The red atoms are polar oxygens, the grey are nonpolar hydrocabons and the yellow are sulphur.

(b) Following the adsorption of the self assembled monolayer at the gold surface a further 8ul of 3mM free lipid in ethanol is allowed to assemble at the surface and then rinsed with buffer(■)

(c) Rinsing causes the mix of tethered and free lipids to form into a tethered bilayer, 4nm thick on a 4nm hydrophilic cushion. The hydrophilic cushion mimics the inside of a cell and the lipid bilayer mimics a cell membrane.

Ion Channels:

Ion channels are molecules that create hydrophilic pathways across lipid bilayer membranes permitting ions to cross otherwise impermeable membranes. Common bacteria such as Pneumonia, Diphtheria, Golden Staphylococcus and Anthrax are pathogenic because the toxins they produce are ion channels that puncture the cells of target organisms and collapse their transmembrane potentials. These toxins usually have large molecular weights in the range 20-50kDa, and assemble into pentamers or heptamers within the membrane that form channels. Figure 2(a) and (b) show the toxin α -hemolysin from the common pathogen *Staphylococcus*. The conductance pathways of membranes containing α -hemolysin is shown in Figure 2. Found commonly in hospitals, Golden Staphylococcus is now essentially immune to penicillin and significant international research effort is being directed to finding a replacement antibiotic.

Hemolysin:

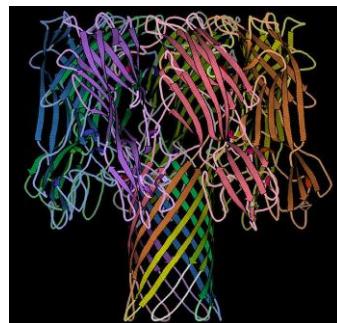
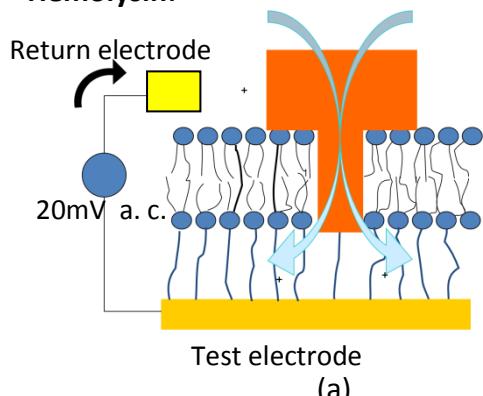


Figure 2.

The measurements of ion currents across a tethered membrane containing α -hemolysin:

(a) An alternating a.c. voltage source applied between a 4mm^2 gold "Return" electrode placed opposite the 2mm^2 "Test" electrode to which the tethered membrane assembly containing α -hemolysin is attached.

(b) The bacterial ion channel toxin α -hemolysin shown schematically in (a). As a pentamer α -hemolysin facilitates ion currents through the normally impermeable lipid bilayer. There is no ion selectivity and all ions positive and negative mono and divalent can pass through α -hemolysin. The conductivity is $\sim 1\text{nS}$.

Another ion channel, found in soil bacteria, is gramicidin A (Figure 3). Being much smaller, with molecular weight of 1882, two molecules end-to-end are required to span the lipid bilayer. Gramicidin is ion selective and is only conducive to monovalent cations. The conductivity to Na^+ is $\sim 1\text{pS}$ in physiological buffer solution (PBS).

Gramicidin:

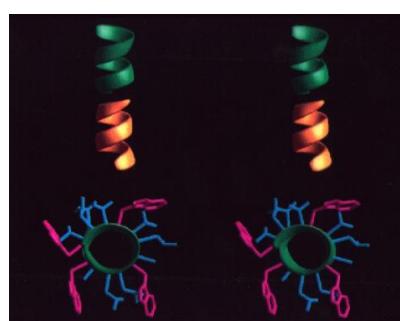
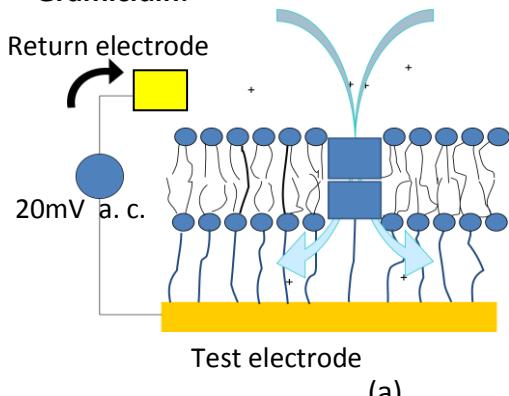


Figure 3.

The measurements of ion currents across a tethered membrane containing gramicidin:

(a) An alternating a.c. voltage source applied between a 4mm^2 gold Return electrode placed opposite the 2mm^2 Test electrode to which the tethered membrane assembly containing gramicidin is attached.

(b) The bacterial ion channel gramicidin. Monomers in the upper and lower leaflets of the bilayer membrane need to align to form a continuous channel to permit ions to cross the membrane. A stereo pair of channel are shown here in both elevation and plane view. The in plane view shows the 0.4nm pore of the gramicidin channel.

Valinomycin:

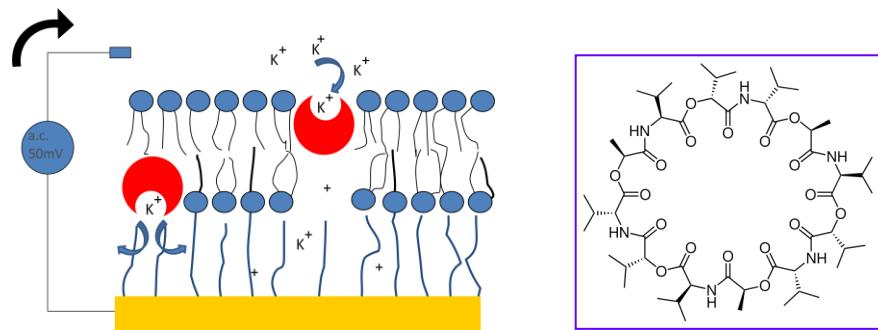


Figure 4.

The measurements of ion currents across a tethered membrane containing valinomycin:

(a) An alternating a.c. voltage source applied between a 4mm² gold Return electrode placed opposite the 2mm² Test electrode to which the tethered membrane assembly containing gramicidin is attached.

(b) The bacterial carrier p[olypeptide valinomycin. Valinomycin complexes with potassium ions in the at the upper and lower leaflets of the bilayer membrane resulting in a concentration driven flow of potassium ions. The complexation of potassium ions with valinomycin is very specific and results in a 1:10,000 specificity against even sodium ions.

How To Make and Measure a Tethered Membrane

The equipment:



Figure 5. The equipment needed to make and measure the conductance of a tethered membrane. Clockwise from top left:

- (i) Pack of cartridge tops with gold counter electrode coated on the reverse face. Also with adhesive coating on the rear face to permit assembly of the flow cell cartridge.
- (ii) "tethaPod" tethered membrane conductance reader.
- (iii) Clamp to compress the electrode onto the adhesive coated cartridge top to make a flow cell test element.
- (iv) Alignment jig to ensure the slide with the patterned gold electrode containing the tethered elements of the tethered membrane are correctly aligned with the cartridge top.
- (v) Test card to demonstrate the reader is functioning correctly.
- (vi) Silicon rubber pressure spreader and aluminium pressure plate to distribute the clamp pressure across the slide and cartridge during the adhesive sealing step.
- (vii) Vials of phospholipid mix including the ion channel gramicidin or valinomycin to add to the monolayer of tethered components on the slide to form a tethered lipid bilayer membrane.
- (viii) A separate vial containing the protein hemolysin can be supplied if required.
- (ix) Individual gold patterned slides coated with a monolayer of the tethered component of membrane stored in metallised sealed packs. These are stored in ethanol for long term shelf life.

Figure 5 shows the equipment to assemble and measure the conductance of a tethered membrane. In addition you will need:

- (i) Pair of scissors to open the slide pack
- (ii) A 10ul and 100ul pipette and tips to deliver the phospholipid(10ul) and rinse with buffer(100ul)
- (iii) Tweezers to remove the slide from the sealed pack.
- (iv) Waste bin to collect used tips.
- (v) Phosphate buffered saline (100ml).
- (vi) Timer to measure 2 minute incubation times for forming the membrane and a one minute delay for the adhesive to seal.

TethaPlate:

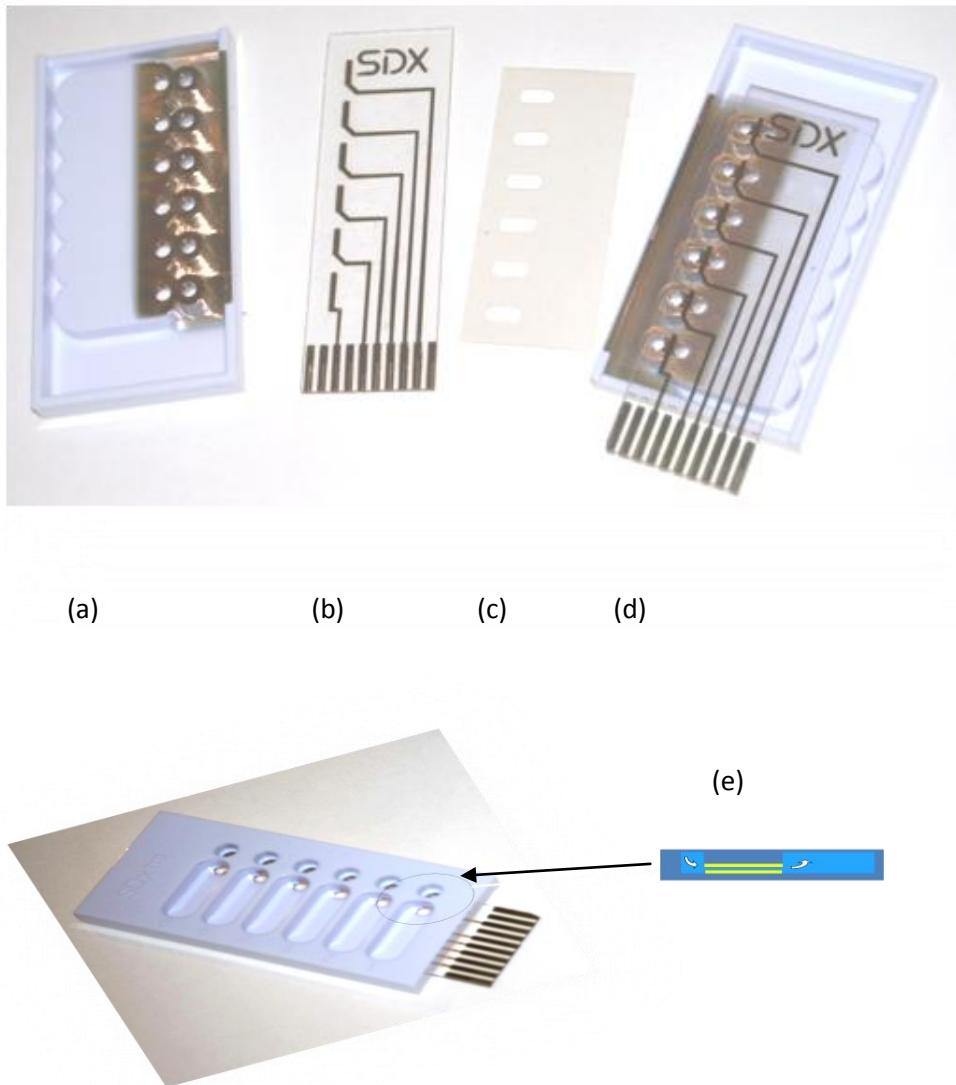


Figure 6.

- (a) The cartridge top viewed from the side coated with the gold counter electrode and adhesive film. The adhesive film has a protective cover sheet which must be removed immediately prior to assembling the flow cell.
(b) The patterned gold slide with six electrode tracks coated with a monolayer of tethered elements of the tethered membrane.
(c) A separate laminate which is attached to the rear of (a) when supplied.
(d) fully assembled slide and cartridge top. (e) When assembled the laminate, the flow cell top and the slide form a flow cell from the round addition opening over the tethered membrane and into the oblong collection reservoir.

Alternating Current (a.c.) Impedance Spectroscopy

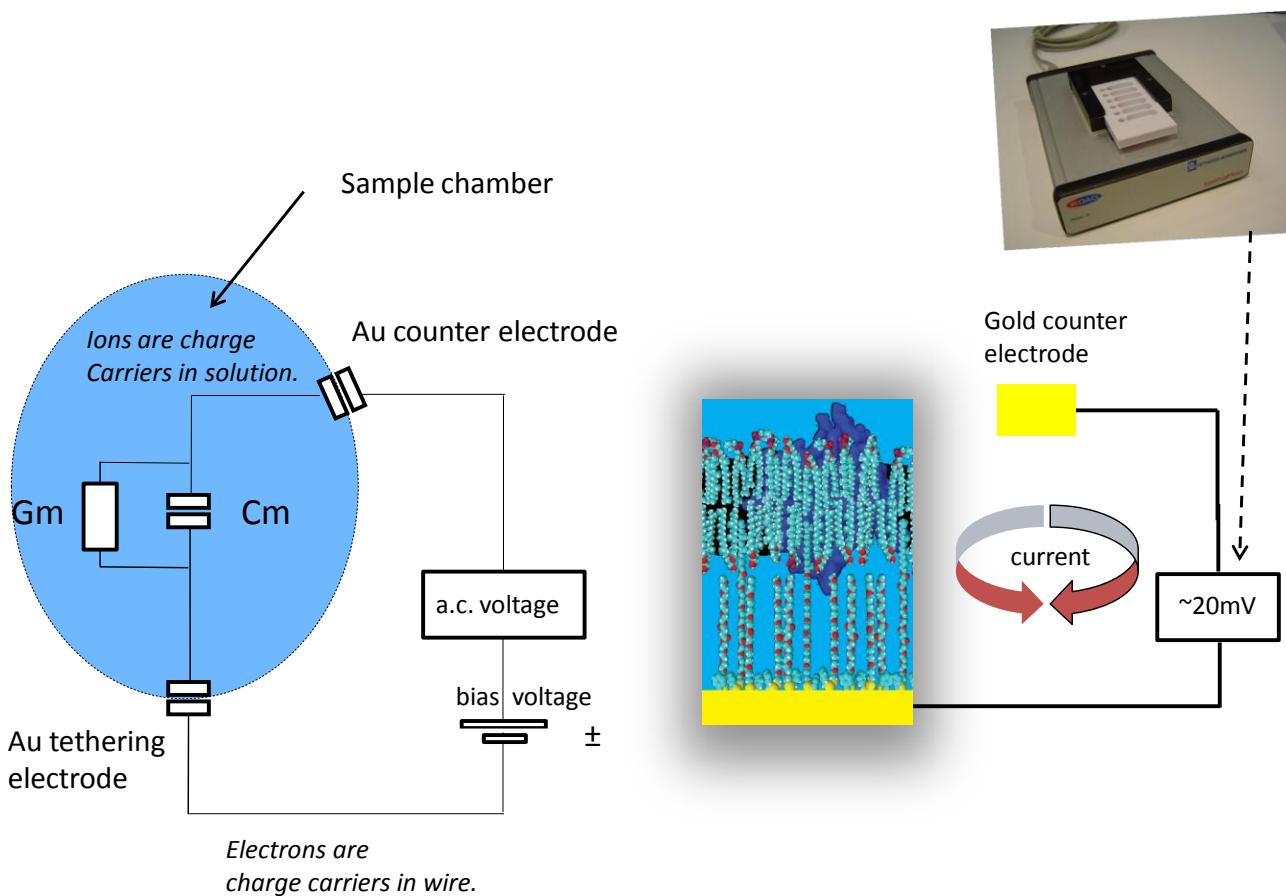


Figure 7. The equivalent electrical elements for the tethered membrane. Important features are the capacitor elements equivalent to the gold tethering electrode and the gold counter electrode. One plate of each of these capacitors represents ions at the gold surface in the ionic solution, and the matching plate in each case is the electronic charge in the gold metal. Because of these coupling capacitors the membrane element of interest, G_m , the membrane conductance due to ion channel currents or lipid leakage, cannot be read using a simple d.c. current meter. Instead it is necessary to use a.c. impedance measurements and resolve the real part of the impedance signal which reports on the current through G_m and the imaginary part which reports on the membrane capacitance C_m . The ThetaPod device used here fits a three capacitor, one conductor model to the experimental data and provides a readout of G_m and C_m , thus avoiding the need to perform the more complex calculations.

The equivalent circuit of a tethered membrane is shown in Figure 6. A sine wave excitation of 20mV is applied across the tethered membrane between the tethering gold electrode and the gold counter electrode. Figure 5 shows these electrodes in the measurement cartridge.

Figure 7 shows the computer screen. The "Setup" menu provides the ability to set a bias voltage (d.c. potential) across the tethered membrane circuit, (+100mV to -100mV). This potential is shown as the battery symbol in Figure 6. Note that this d.c. potential only charges the coupling capacitors at the tethering gold surface and at the counter electrode. No potential is applied across the membrane elements G_m and C_m . The "Chart" menu permits a choice of variables to display as a function of time on the graphical trace or a numerical DVM (digital voltmeter) display. Also the "Table" menu permits a choice of variables to show in the tabulation at the bottom of the display. GoF is an acronym for "goodness of fit" which is a measure of the match between the modelled spectrum for the displayed G_m and C_m and the

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experimental data. GoF values should be less than 0.1 to validate the model. Should the GoF be greater than 0.15 it means the the tethered membrane is not capable of being modelled by this equivalent circuit and the readings of Gm and Cm values should be disregarded.

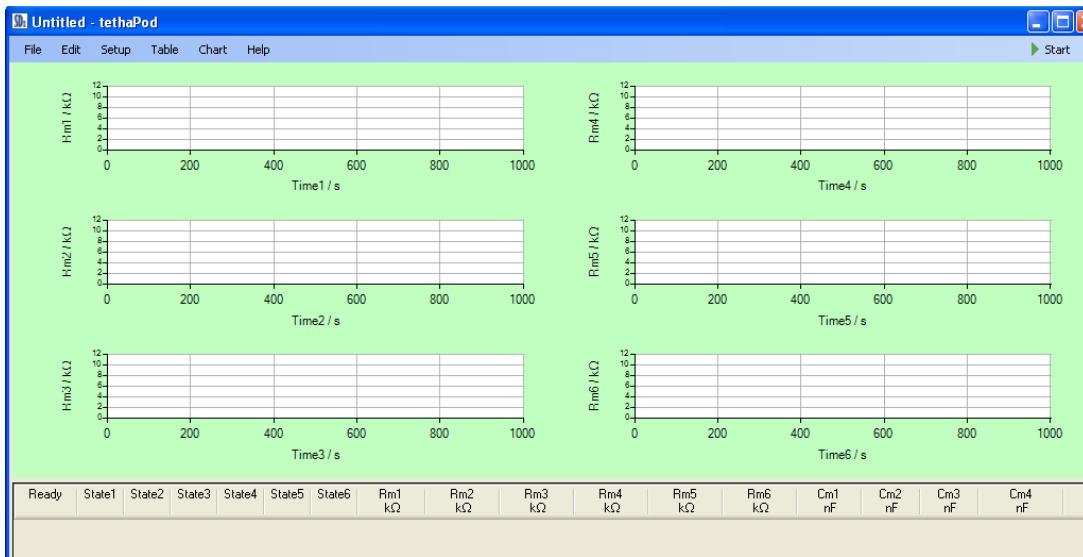


Figure 8. The chart display on the conductance reader. The menu at the top of the display includes “Setup” which allows the bias voltage to be set, “Table” which allows selection of variables to be displayed in the tabulation at the bottom of the “Chart” page. GoF and “State” are included in the “Table” menu. “State” indicates the stage at whih the fitting program has reached in mimicking the experimental data.



Figure 9. The chart readout display on the conductance reader. The menu at the top of the display includes “Setup” which allows the bias voltage to be set, “Table” which allows selection of variables to be displayed in the tabulation at the bottom of the “Chart” page. GoF and “State” are included in the “Table” menu. “State” indicates the stage at whih the fitting program has reached in mimicking the experimental data.

Introduction to practical exercise 1

Aim:

1. To prepare tethered membranes containing gramicidin A (gA).
2. To measure the membrane conductance.
3. Measure the conductance dependence on gramicidin concentration. Use this measurement to calculate :
 - a. the conduction of a dimeric gramicidin channel
 - b. the concentration of gramicidin required to form a dimer.
4. Determine the dependence of conductivity on the bias potential and from this determine the ion selectivity.

Materials supplied:-

Six lipid solutions

Vial 1: 3mM Phospholipid, 0 nM gA
Vial 2: 3mM Phospholipid, 80 nM gA
Vial 3: 3mM Phospholipid, 110 nM gA
Vial 4: 3mM Phospholipid, 140 nM gA
Vial 5: 3mM Phospholipid, 170 nM gA
Vial 6: 3mM Phospholipid, 200 nM gA

Procedure:

Ensure all equipment, instrumentation and chemicals are available when you start. Timing is critical for proper membrane formation. Read the entire experiment through before commencing.

Exercise 1(1). Prepare tethered membranes containing Gramicidin

- a. Cut open the silver foil pack, and using tweezers remove the slide.
- b. *(Never touch the slide with bare hands. Never touch the gold with fingers as this may damage the lipid coating lipid formation of the membrane.)*
- c. The electrode is stored in ethanol and you need to stand it on a tissue to dry. This may take 1-2 minutes.
- d. Align the dry slide over the alignment jig, ensuring electrode tracks and the SDX logo on the slide overlay each other. Using tweezers gently push electrode into the slot.
- e. Remove top layer of plastic from the cartridge. This will reveal a sticky surface which will then bind to the electrode upon contact.
- f. Position white cartridge over the top and push into position. Once the two surfaces meet do not peel them apart or attempt to re-locate them as it will damage the electrode.
- g. Gently put the cartridge and electrode into the clamp and tighten. Allow to stand for at least 30 seconds, before loosening pressure. The “tethaPlate” is now ready for membrane formation.

Membranes are formed as follows:

- g. Start stop watch.
- h. Add 8 μ L phospholipid solution (no gA) to chamber 1.
- i. At 15 seconds, add 8 μ L 80nM gA solution to chamber 2.
- j. At 30 seconds, add 8 μ L 110nM gA solution to chamber 3.
- k. At 45 seconds, add 8 μ L 140nM gA solution to chamber 4.
- l. At 60 seconds, add 8 μ L 170nM gA solution to chamber 5.
- m. At 75 seconds, add 8 μ L 200nM gA solution to chamber 6.
- n. At 120 seconds, to chamber 1 add 100 μ L PBS.
- o. At 135 seconds, to chamber 2 add 100 μ L PBS.
- p. At 150 seconds, to chamber 3 add 100 μ L PBS.
- q. At 165 seconds, to chamber 4 add 100 μ L PBS.
- r. At 180 seconds, to chamber 5 add 100 μ L PBS.
- s. At 195 seconds, to chamber 6 add 100 μ L PBS. (Total 3 minutes, 15 seconds elapsed).

Chamber	Constituent in Phospholipid	
1	No gA	[vial 1]
2	80nM gA	[vial 2]
3	110nM gA	[vial 3]
4	140nM gA	[vial 4]
5	170nM gA	[vial 5]
6	200nM gA	[vial 6]

Label the adhesive foil cover and with your name and the date. Remove its protective film and stick on the tethaPlate to cover the chambers.

Exercise 1(2). Measure the conductance and capacitance of tethered membranes.

- a. Insert the tethaPlate cartridge into the TethaPod.
- b. Open “TethaPod” Software. A green LED lights on the front panel of the tethaPod when the instrument is working properly. Examine the menus *Table*, *Setup*, *Graphs*.
- c. Set: “GoF” (*goodness of fit*) to 0.12 (*Table/Set GoF Threshold*)
- d. Set the potential bias to 100mV. (*Setup/Set Bias*)
- e. Set instrument to show Gm. (*Table/✓Gm*).
- f. Press “Start”.
- g. The instrument will measure the membrane conductance from each chamber sequentially. (The instrument is actually fitting a complex impedance function from the sample at a range of frequencies from 1kHz to 0.1Hz. To avoid the user having to deal with complex impedances the instrument fits capacitance and conductance values to the data.)
- h. Once all channels read “Yes” (Ready column) stop recording.
- i. Save data into an Excel Spreadsheet. [Edit/copy selection/paste into Excel/save spreadsheet].
- j. Set new bias at -100mV. Wait until reader stabilises. Repeat steps e through i (do not save the file when asked at step e).
- k. Set new bias at +100mV. Repeat steps e through i (do not save the file when asked at step e).

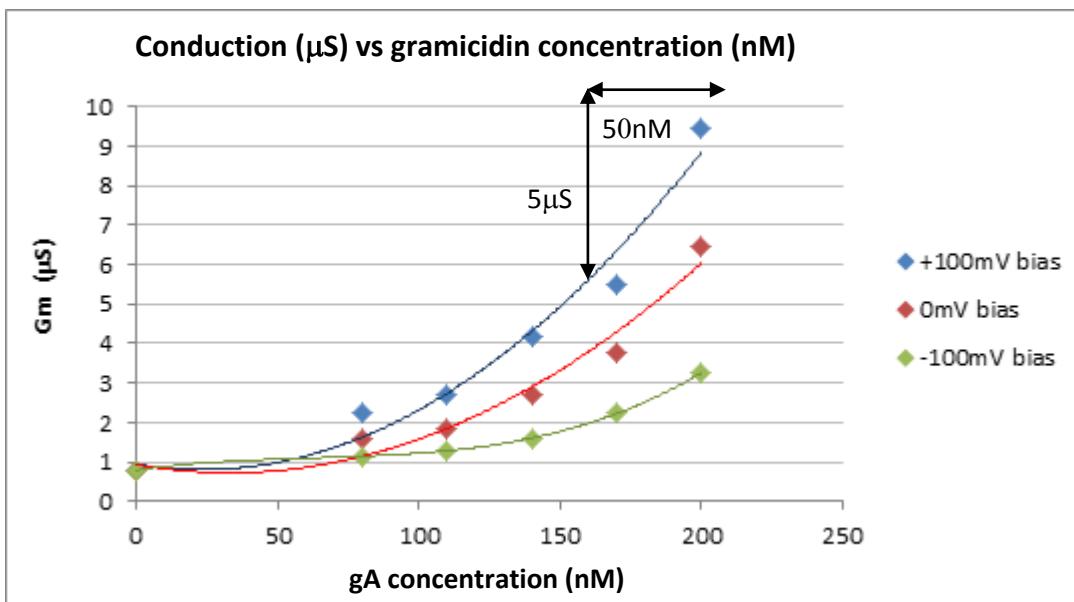
Exercise 1(3). Writing Report:

Tabulation:

From the data you recorded, generate a Table of conduction (G_m in μS) versus gramicidin concentration ([gA] in nM), for 0, 100mV and -100 mV bias. An example is given as Table 1 below.

Table1:

Channel	gA (nM)	G_m (0mV bias)	G_m (-100mV bias)	G_m (+100mV)
1	0	0.792	0.791	0.759
2	80	1.608	1.150	2.263
3	110	1.865	1.275	2.702
4	140	2.701	1.594	4.164
5	170	3.751	2.246	5.499
6	200	6.459	3.251	9.468



Questions:

- (i) Describe the effect of applying a positive or negative bias potential from the outer to inner surface of the membrane. I.e. positive to the outer surface means negative on the tethering gold relative to counter electrode.

What is an explanation for this effect?

{Answer: The channel is cation selective.}

(ii) Calculate Conduction per gA Channel

1. Estimate the maximum slope obtained for graph of conduction vs gramicidin concentration. Mark on your graph how this was obtained. i.e. $5\mu\text{S}$ for 50nM gA (+100mV bias).

2. Calculate the number of phospholipid molecules we added to each cell to make the tethered membrane.

Molarity of phospholipid = 3mM

Volume added = $8\mu\text{L}$

$$\begin{aligned}\text{Molecules} &= \text{Molarity (mol/L)} \times \text{Volume (L)} \times \text{Avogadro's number (molecules/mol)} \\ &= 3 \times 10^{-3} \text{ mol/L} \times 8 \times 10^{-6} \text{ L} \times 6.2 \times 10^{23} \text{ molecules/mol} \\ &= \underline{\underline{1.488 \times 10^{16} \text{ molecules}}}\end{aligned}$$

3. Calculate the number of molecules of phospholipid in tethered monolayer film on gold.

Area per tethered molecule = 1nm^2

Area of gold electrode = $2\text{mm}^2 = 2 \times 10^{12} \text{ nm}^2 = 2 \times 10^{12} \text{ tethered molecules.}$

4. Fraction of added phospholipid incorporated into membrane

$$= 2 \times 10^{12} \text{ tethered molecules} / 1.488 \times 10^{16} \text{ molecules}$$

$$\underline{\underline{\sim 10^{-4} \text{ of the added phospholipid.}}}$$

Note: this tells us that most of the added material is flushed away and only 1/10,000 remains trapped in the membrane.

5. Calculate the number of molecules of gramicidin in $8\mu\text{l}$ of 50nM .

$$\begin{aligned}\text{Molecules moles} &= \text{Molarity (M)} \times \text{Volume (L)} \times \text{Avogadro's number (molecules/mole)} \\ &= 50 \times 10^{-9} \text{ M} \times 8 \times 10^{-6} \text{ L} \times 6.2 \times 10^{23} \\ &= \underline{\underline{2.41 \times 10^{+10} \text{ molecules}}}\end{aligned}$$

6. Assume the same fraction of gramicidin enters the formation mix of the membrane as the fraction of lipids (*they are very similar molecular weights*) then the number of gramicidin in the membrane

$$= 2.41 \times 10^{+10} \times 10^{-4}$$

$$\underline{\underline{\sim 2 \times 10^6 \text{ molecules}}}$$

7. Calculate the conductance generated per gramicidin (in pS).

$$\begin{aligned}\text{Siemens per ion channel} &= \text{Total Siemens generated} / \text{number of gramicidin molecules} \\ &= 5.00 \times 10^{-6} \text{ S} / 2.41 \times 10^{+6} \text{ molecule} \\ &= \underline{\underline{\sim 2 \text{ pS/molecule}}}\end{aligned}$$

{**Answer:** The published result from complex single channel measurements estimates 1pS . This provides a surprisingly simple estimate of the order of magnitude of molecular conduction.}

(iii) Calculate the thickness of the lipid bilayer tethered membrane.

From the Chart menu select Cm and DVM. Read value for each chamber.

Calculate membrane thickness from the relationship between area plate separation and permittivity of a capacitor:

i.e. for a capacitor of area, A (m^2) and thickness, d (m) and capacitance, Cm (F) is given by: $Cm = \epsilon_0 \times \epsilon_r \times A / d$, where ϵ_0 is the permittivity of free space = 8.854×10^{-12} F/m and ϵ_r is the relative permittivity of membrane lipid ~ 2.3 and area A = 3mm^2

{**Answer:** Cm $\sim 4\text{nm}$.}

(iv) why is the membrane becoming thinner when more gA is added?

{**Answer:** because gA is shorter than the membrane lipid}

Introduction to practical exercise 2

Aim:

1. To prepare tethered membranes containing Valinomycin (Val).
2. To measure the membrane conductance.
3. Measure the conductance dependence of Val for Na^+ and K^+ . Use this measurement to calculate:
 - a. the ratio of the conductance of Val to gA.
 - b. the specificity of Val for K^+ .

Materials supplied:-

Two lipid solutions	Vial 1: 3mM Phospholipid, 0 nM Val
	Vial 2: 3mM Phospholipid, 0.9mM Val
	Test tube 1 PBS
	Test tube 2 PBS + 1mM KCl
	Test tube 3 PBS +10mMKCl

Procedure:

Ensure all equipment, instrumentation and chemicals are available when you start. Timing is critical for proper membrane formation. Read the entire experiment through before commencing.

Exercise 2(1). Prepare tethered membranes containing Valinomycin

- h. Cut open the silver foil pack, and using tweezers remove the slide.
- i. (*Never touch the slide with bare hands. Never touch the gold with fingers as this may damage the lipid coating lipid formation of the membrane.*)
- j. The electrode is stored in ethanol and you need to stand it on a tissue to dry. This may take 1-2 minutes.
- k. Align the dry slide over the alignment jig, ensuring electrode tracks and the SDX logo on the slide overlay each other. Using tweezers gently push electrode into the slot.
- l. Remove top layer of plastic from the cartridge. This will reveal a sticky surface which will then bind to the electrode upon contact.
- m. Position white cartridge over the top and push into position. Once the two surfaces meet do not peel them apart or attempt to re-locate them as it will damage the electrode.
- n. Gently put the cartridge and electrode into the clamp and tighten. Allow to stand for at least 30 seconds, before loosening pressure. The “tethaPlate” is now ready for membrane formation.

Membranes are formed as follows:

- z. Start stop watch.
- aa. Add 8 μ L phospholipid solution to chamber 1.
- bb. At 15 seconds, add 8 μ L phospholipid solution to chamber 2.
- cc. At 30 seconds, add 8 μ L phospholipid solution to chamber 3.
- dd. At 45 seconds, add 8 μ L phospholipid solution (plus 0.9M Val) to chamber 4.
- ee. At 60 seconds, add 8 μ L phospholipid solution (plus 0.9M Val) to chamber 5
- ff. At 75 seconds, add 8 μ L phospholipid solution (plus 0.9M Val) to chamber 6.
- gg. At 120 seconds, to chamber 1 add 100 μ L PBS.
- hh. At 135 seconds, to chamber 2 add 100 μ L PBS.
- ii. At 150 seconds, to chamber 3 add 100 μ L PBS.
- jj. At 165 seconds, to chamber 4 add 100 μ L PBS.
- kk. At 180 seconds, to chamber 5 add 100 μ L PBS.
- ll. At 195 seconds, to chamber 6 add 100 μ L PBS. (Total 3 minutes, 15 seconds elapsed)

Cell	Constituent in Phospholipid	
1	No Val	[vial 1]
2	No Val	[vial 1]
3	No Val	[vial 1]
4	0.9mM Val	[vial 2]
5	0.9mM Val	[vial 2]
6	0.9mM Val	[vial 2]

Label the adhesive foil cover and with your name and the date. Remove its protective film and stick on the tethaPlate to cover the chambers.

Exercise 2(2). Measure the conductance and capacitance of tethered membranes.

- i. Insert the tethaPlate cartridge into the TethaPod.
- m. Open “TethaPod” Software. A green LED lights on the front panel of the tethaPod when the instrument is working properly. Examine the menus *Table*, *Setup*, *Graphs*.
- n. Set: “GoF” (*goodness of fit*) to 0.12 (*Table/Set GoF Threshold*)
- o. Set the potential bias to 100mV. (*Setup/Set Bias*)
- p. Set instrument to show Gm. (*Table/✓Gm*).
- q. Press “Start”.
- r. The instrument will measure the membrane conductance from each chamber sequentially. (The instrument is actually fitting a complex impedance function from the sample at a range of frequencies from 1kHz to 0.1Hz. To avoid the user having to deal with complex impedances the instrument fits capacitance and conductance values to the data.)
- s. Once all channels read “Yes” (Ready column) record Gm values and:
 - a. flush chamber 1 with 100ul PBS
 - b. flush chamber 2 with 100ul PBS plus 1mM potassium chloride
 - c. flush chamber 3 with 100ul PBS plus 1mM potassium chloride
 - d. flush chamber 1 with 100ul PBS
 - e. flush chamber 2 with 100ul PBS plus 1mM potassium chloride
 - f. flush chamber 3 with 100ul PBS plus 1mM potassium chloride
- t. Once the conductance trace has settled record stop recording.
- u. Save data into an Excel Spreadsheet. [Edit/copy selection/paste into Excel/save spreadsheet].

Exercise 2(3). Writing Report:

Tabulation:

From the data you recorded, generate a Table of conduction (G_m in μS) versus gramicidin concentration ($[gA]$ in nM), for 0, 100mV and -100 mV bias. An example is given as Table 1 below.

Table1:

Chamber	Ionophore Conc.	Ionic Solution
1	0 Val	PBS
2	0 Val	PBS + 1mM KCL
3	0 Val	PBS + 10mM KCL
4	0.9mM Val	PBS
5	0.9mM Val	PBS + 1mM KCL
6	0.9mM Val	PBS + 10mM KCL

Questions:

- (i) Describe the effect of the addition of small amounts of KCl to the PBS. What is an explanation for this effect?

{**Answer:** The channel is strongly potassium ion selective. The ratio of Na^+ to K^+ conductance can be >1:1000.}

- (ii) Calculate the ratio of the Conduction of Val Ionophore compared to the gA in exercise 1.

{**Answer:** Evident from the nM concentrations of gA added in exercise 1 to mM concentrations of Val added in exercise 2.}

- (iii) Discuss why channels are more efficient at ion transport than carriers.

{**Answer:** One channel can transport many ions per second through a permanent opening. A carrier can only transport one ion per carrier complexing, transporting and releasing an ion.}