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enabling technologies for Life Science

How to get started with lipid bilayer experiments

Goal of the guide:

help novices of lipid bilayer experiments to form a lipid membrane in the BLMchip.

Material overview:

The type of lipid, organic solvent and electrolyte solution suggested in this guide are all proven by different users and therefore represent an excellent starting point for a basic experiment to help novices to familiarize with the device. Remember that the type of lipid, solvent, and electrolyte solution we use here are not the only conditions under which the membrane can be formed, but we suggest you start from these indications to get familiar with the device.

Please note:

If you have difficulty forming membranes with your approach, follow this step-by-step guide so that you are using a proven method that ensures the formation of good lipid membranes. If after scrupulously following all the steps in this guide you are still unable to form a membrane, please contact us by email.

Material needed and reagents preparation:

BLMchip 150 µm (provided by Elements). BLM chips are available with two different sized holes (100 µm, 150 µm). For a neophyte, 150 µm is best size to start with, as it is less rare to form lipid plugs.

DPhPC lipids (Avanti, CAS number 207131-40-6). We suggest starting with the powdered form as it is the easiest to prepare. Weigh a small amount (about 5 mg) of lipid powder and add the required volume of solvent to reach 10 mg/ml in a small tube (preferably, use glass-made tubes for this purpose). You can use a vortex or a shaker to help the lipids dissolution. Aliquots of dissolved lipids can be stored at -20°C for many days. It is recommended to parafilm the lid of the tube to prevent the solvent evaporation.

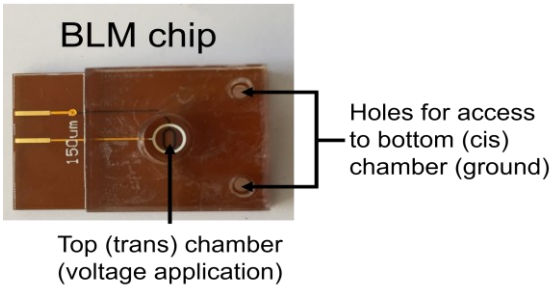
Organic solvent needed to dissolve the lipids: n-Hexane

Electrolyte solution: 3M KCl, 20mM TRIS, PH 8. Filter at 0.2 µm.

Pore forming protein: we suggest using the alpha hemolysin (Sigma-Aldrich, H9395-.5MG), a good protein to start with as it inserts quite easily into the bilayer and it is water soluble. The use of the pore forming protein is recommended but not mandatory to learn how to paint the membrane. Alpha-hemolysin preparation: dissolve the powder in milliQ water to obtain a stock

solution 0.1 mg/ml concentrated. Add 1 µl of the stock solution in 500 µl of the electrolyte solution; this quantity is sufficient for many experiments. Both the stock solution and the dilution must be stored at + 5 °C. The stock is stable for several months.

Step-by-step guide:



Step 1_Chlorinate the silver electrodes

Chlorinate the Ag electrodes on the BLM chip by adding 60 µl of pure bleach to the top (trans) chamber and 50 µl of bleach to the bottom (cis) chamber through one of the two holes. Check the color of the electrode: when properly chlorinated, from silver must turn to black, as shown in the figure below (approximately 10 min). Make sure you are not using an old bleach as the chlorine content might be diminished. Afterwards, rinse with 70% EtOH and finally with abundant ddH_2O (a lab wash squeeze bottle is best suited to finely clean the chambers). If possible, dry the two compartments with a gentle flow of air. Alternatively, use a pipette to take out any residual ddH_2O, especially from the bottom compartment.

Before chlorination

After chlorination

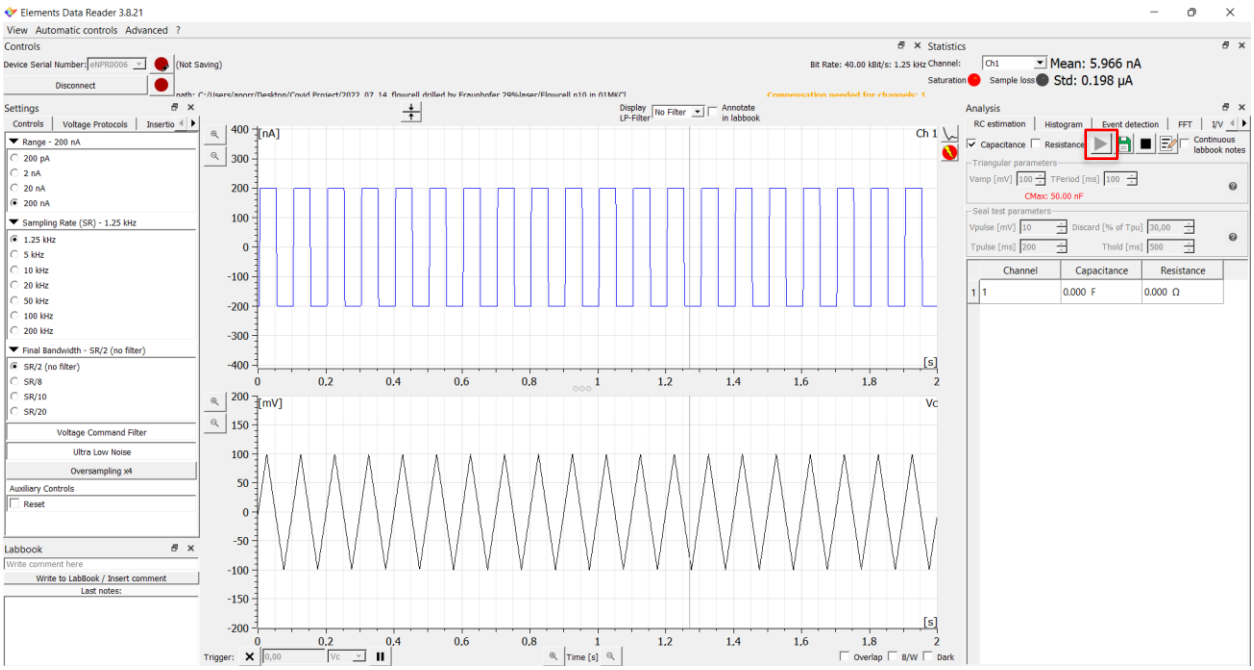
Step 2_Fill the BLMchip

Slowly fill the BLM chip with the electrolyte solution by adding 60 µl to the top (trans) chamber and 50 µl to the bottom (cis) chamber through one of the two holes. Insert the chip into the reader.

During the first few usages it is recommended to fill the BLM chip chambers before inserting it into the device to avoid any possible buffer leakages that would harm the electronics.

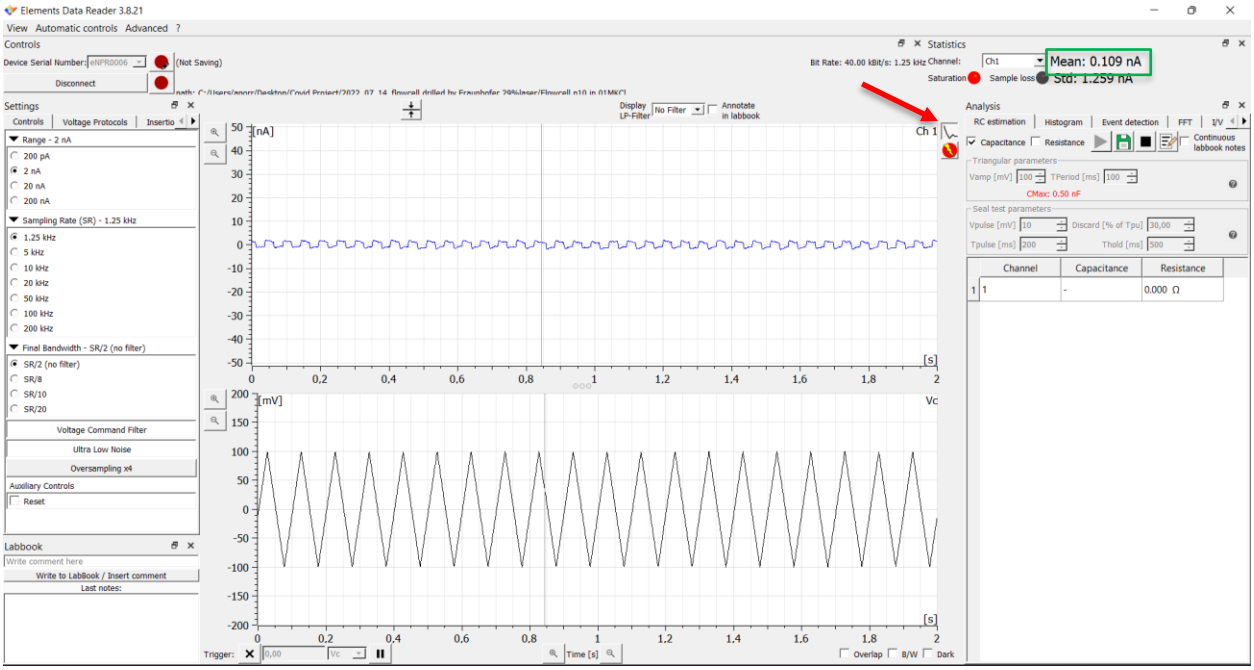
Step 3_Get the pore wet

Uncheck the resistance checkbox in the RC estimation widget and click the display button (red square in the image below) to apply a triangular wave stimulus. Check the proper filling of the pore: a full-scale square wave (due to amplifier saturation) should be seen at the ± 200 nA current range, as shown in the figure below. If not, pipette up and down the electrolyte solution until the pore get wet.



Step 4_Compensate the voltage offsets

Select the 2nA current range and compensate voltage offsets by clicking the “Voltage offset compensation” button (indicated by the red arrow in the image below). When the “mean” value (green square in the image) falls below 0.1 nA, click again the button to disable the tool before proceeding to the next step, otherwise any signal displayed in the oscilloscope window would be meaningless. Note: the current value never stabilizes precisely at the 0 pA value due to the very small resistance of the circuit in this configuration as the ionic current flows through a huge hole (150 µm).

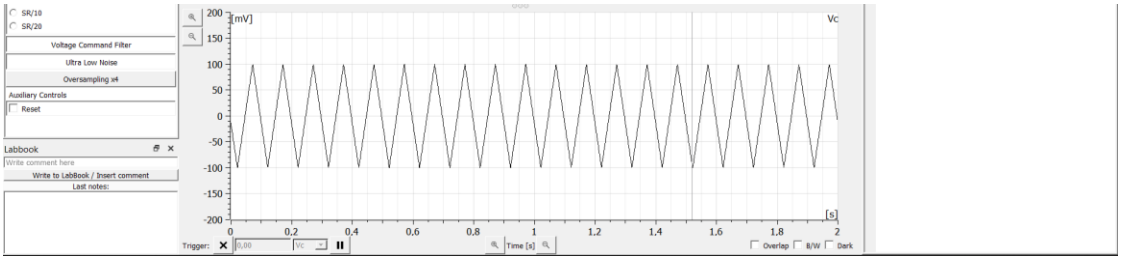


Step 5_Paint the membrane

Paint the membrane using the bubble method, as described below and as explained in the product website page video.

Dip the tip of a p10 pipette in the lipids solution without pulling any liquid. Position the tip in close proximity of the hole and create an air bubble that must spread over the hole. If done correctly, lipid forming the bilayer membrane cause the current response to become square in shape, gaining capacitive features. For novices, the critical part is to recognize a real membrane from a lipid plug. Below are some examples of lipid plugs (panels B,C,D,E) and well-formed lipid bilayer membranes (panels F,G,H,I,J) using the same stimulus protocol (panel A). Panel K shows an example of a leaky membrane; in that case, note that the current response waveform acquires a triangular (resistive) component, which is absent in the well-formed membranes. Overall, the capacitance value of lipid plugs is always significantly lower than that of lipid membranes (check the values indicated by the red arrows) and the shape of the current response is slightly different as it tends to be less square. Typical capacitance values of the membranes formed in our BLMchip are shown in the latest page of this guide. Lipid plugs can also be easily identified using the ZAP tool (click the button indicated by the green arrow below): the short high-voltage stimulus (1.5 V) provided by the ZAP tool is sufficient to break the bilayer membranes but does not do anything to the lipid plugs. If a lipid plugs forms, tap the BLMchip until you see that the plug breaks (the current response must return as in Step 3). If nothing happens, increases the vigor of the tap until the plug breaks down.

A



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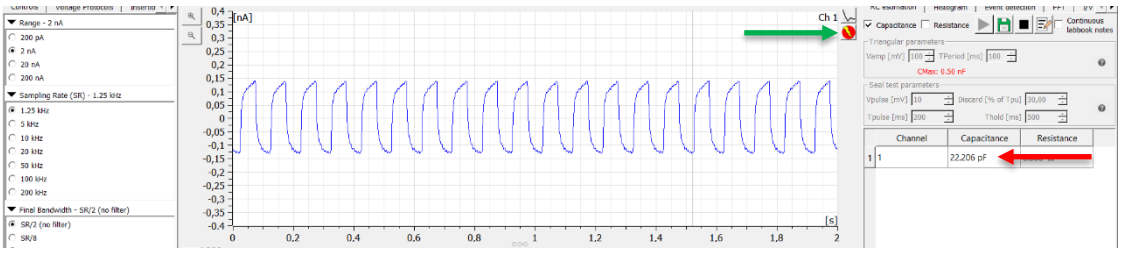

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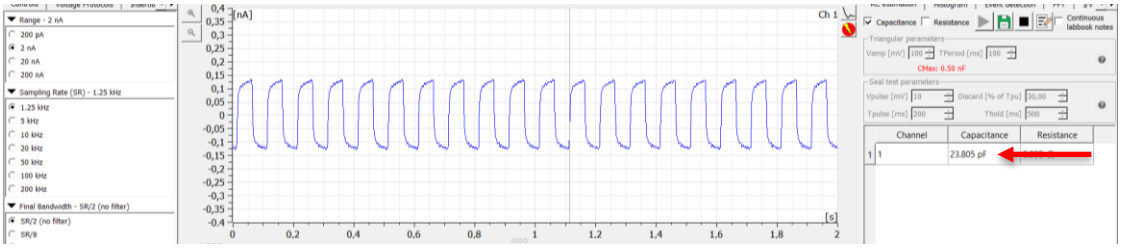

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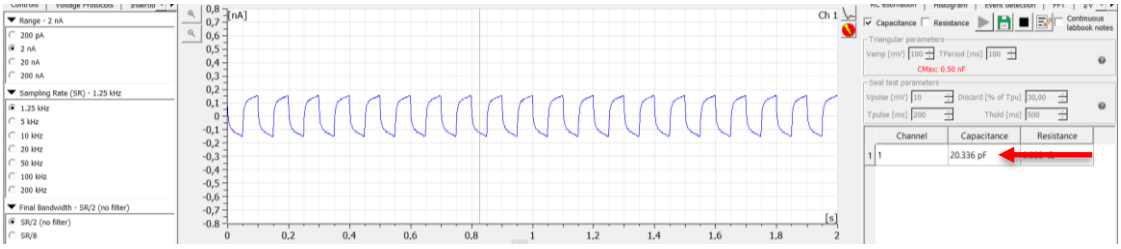
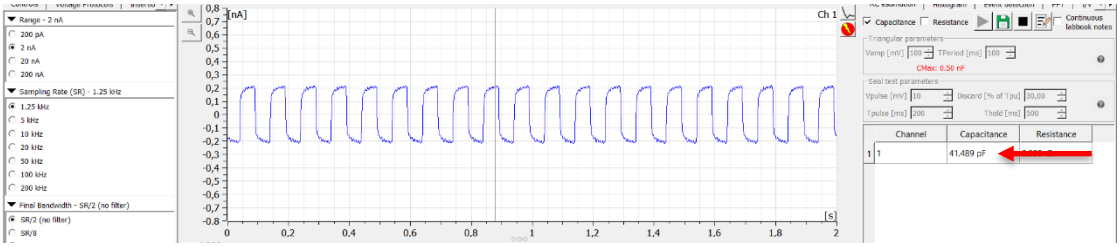


E



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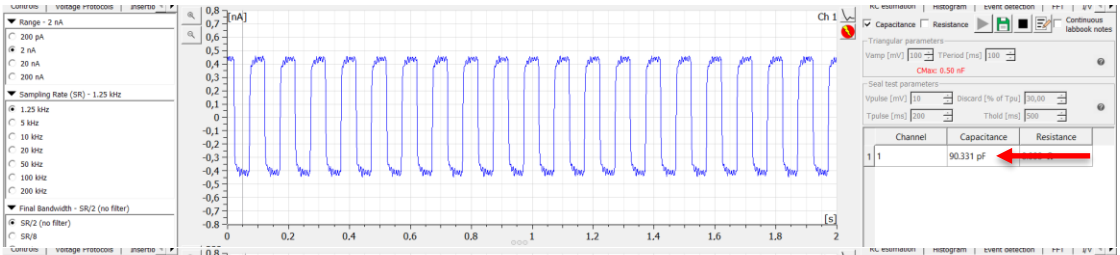
F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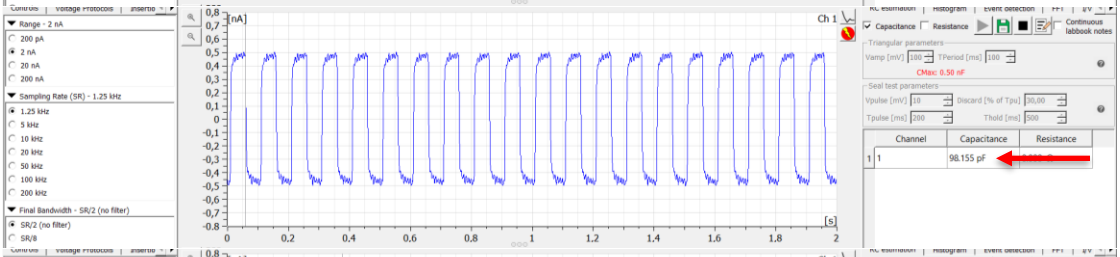
Ch 1
[s]
Capacitance
Resistance
Channel
Capacitance
Resistance
1 1
90.331 pF

G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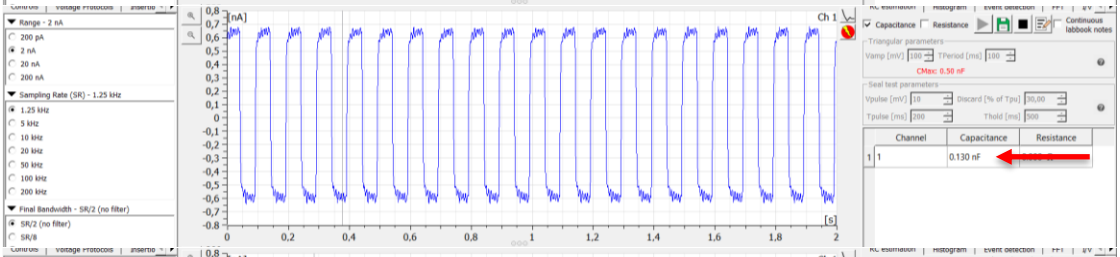
Resistance
Channel
Capacitance
Resistance
1 1
98.155 pF

H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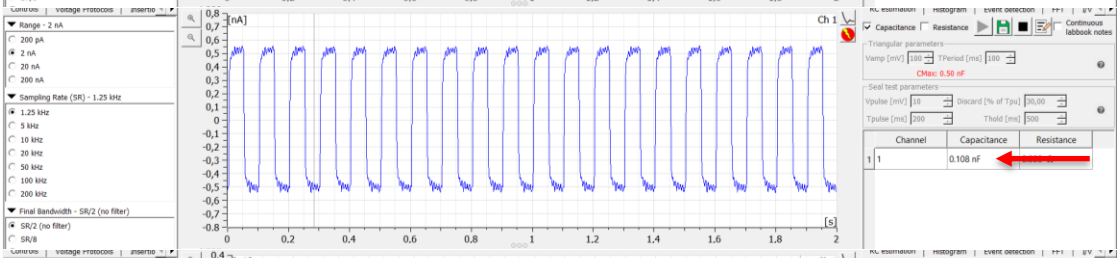
Capacitance
Resistance
Channel
Capacitance
Resistance
1 1
0.130 nF

I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1 1
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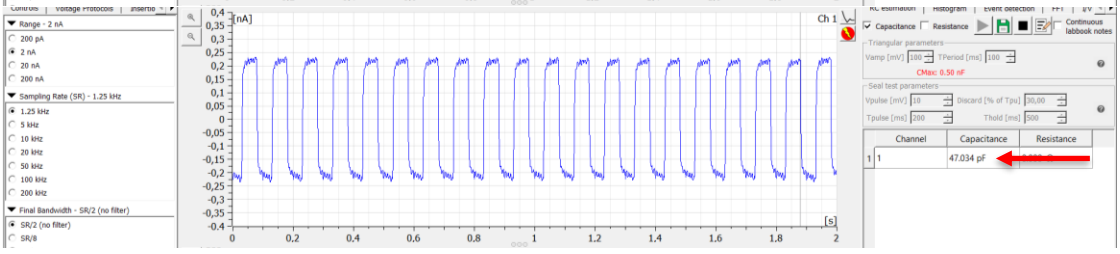
J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Ch 1
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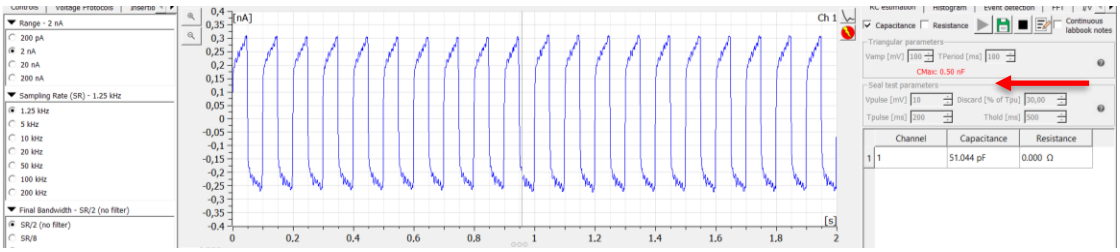
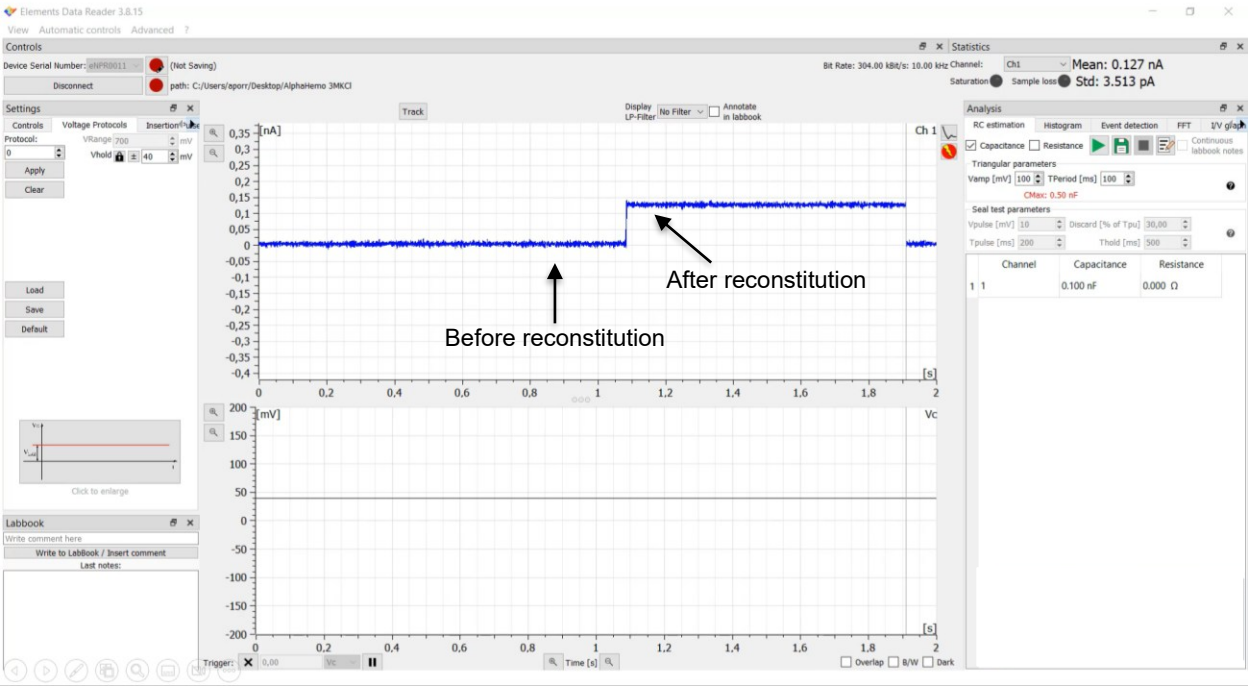
1 1
47.034 pF

K

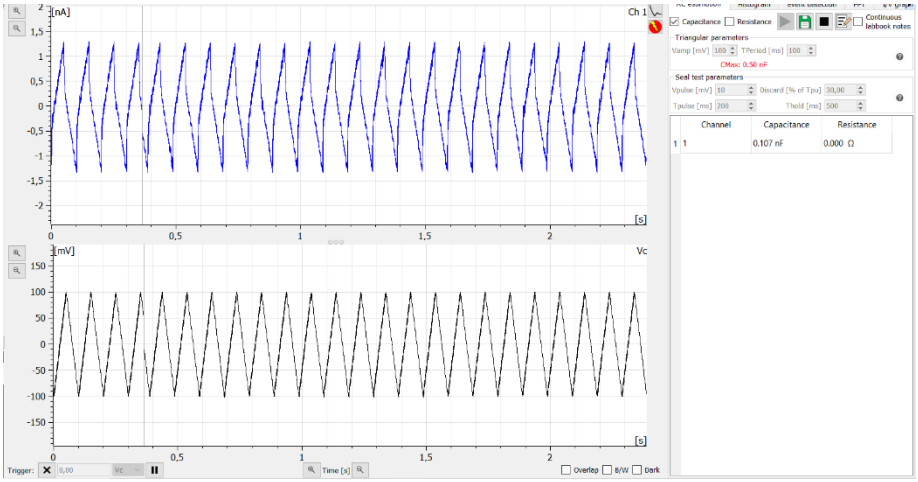

[nA]
Ch 1
[s]
Capacitance
Resistance
Channel
Capacitance
Resistance
1 1
51.044 pF
0.000 Ω

Step 6_Add the pore forming protein

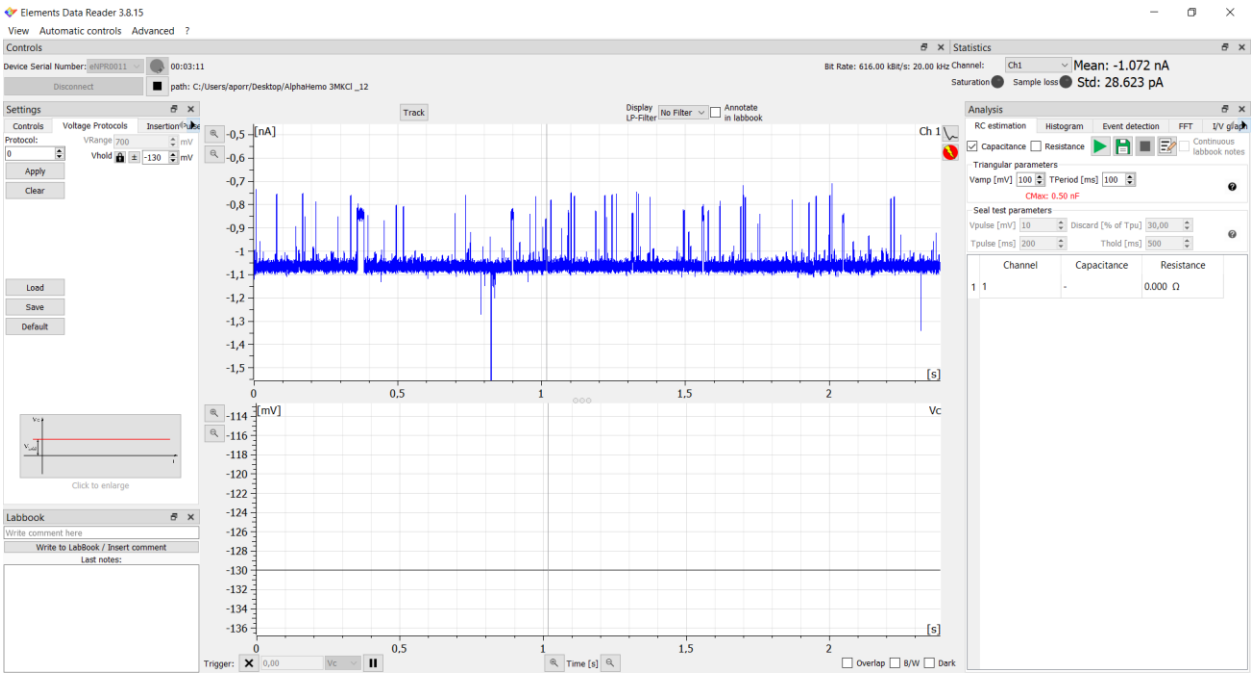
After getting a good membrane, apply a + 40 mV constant voltage stimulus (protocol 0 among the voltage protocols list). Check for the membrane/signal stability for 1 min in these conditions. Add 1ul of the 1:500 dilution (prepared as described in the first page) to the top chamber and gently close the lid of the device. The successful reconstitution of a single channel into the lipid membrane, is visible as a jump of the current signal from 0 to about 125 pA (see image below). Be patient as from the addition of the protein it may take several minutes before you see the first insertion. If after 5 min nothing happens, add another microliter of diluted protein, and wait again. If you still see nothing, break the membrane using the ZAP tool and paint a new one. Adding the protein is no longer necessary since it is already present in the BLMchip chamber.



It may happen that the protein inserts just after the membrane painting. This causes an expected dramatic change in the current response waveform (see image below), as a resistive component (the alpha hemolysin) inserts into the RC circuit.



After getting a single alpha hemolysin pore insertion, you may also add PEG molecules and measure their translocation through the pore (see image below). Add 1ul of a 0.5 mM PEG 1500 solution (in the electrolyte solution) to the top and cis chamber. The translocation should start immediately after the molecule addition. You may also add the PEG molecules at the beginning of the experiment, directly into the electrolyte solution, before filling the BLMchip.



Step 7_Clean the BLMchip

When finished, rinse both chambers first with ddH_2O, then with Ethanol or Isopropanol, and then again with abundant ddH_2O. A lab wash squeeze bottle best suited to finely clean the chambers. Finally, a gentle flow of compressed air is recommended to completely dry the chambers.

Additional information

- Typical membrane capacitance in the BLMchip: 20-50 pF in BLMchip 100 µm | 50-110 pF in BLMchip 150 µm.
- Usage of the eNPR over a long period of time can lead to an increase in temperature within the BLM chips (~30-31 °C). Please keep in mind that this might influence the ion channel/porins activity.
- To improve the BLM quality while painting, additional optical support can be used (binoscope/microscope) to check for possible air bubbles.
- The lid should be applied gently to the device to avoid breakage of the BLM.

For a more accurate guide on BLM experiments and tips, please visit our website (Products → BLM kit → How to) for the BLM Kit Quick Guide or the BLM Application note.

Troubleshooting and additional tips

- If the first membranes formed show a resistive component—easily recognizable by the triangular shape of the current response (as described above)—it may help to completely remove the electrolyte from the upper compartment (trans chamber) and reintroduce it after a few seconds. Alternatively, the withdrawn electrolyte can be replaced with fresh solution.

- In several cases, membrane formation does not occur immediately. It may be necessary to apply multiple bubbles before obtaining a signal characteristic of a lipid bilayer.

- After immersing the tip into the lipid solution, immediately proceed with the formation of the bubble at the micrometric aperture on the BLM chip, since the solvent evaporates quickly. Each time the tip is dipped into the lipid solution, it must be replaced to avoid contaminating the lipid solution with water from the experimental electrolyte.

- If, after many attempts, no membrane formation is observed, clean the chip with isopropanol and rinse it with water as described in the “Clean the BLM chip” section. After carefully drying the chip (it must be completely dry), dispense approximately 1.5 µL of a solution containing 1% hexadecane in n-hexane onto the aperture. This treatment makes the region around the

aperture more hydrophobic, promoting lipid adhesion. Wait about one minute for complete solvent evaporation, then add the electrolyte and proceed with membrane formation using the bubble method, as described above. This treatment is recommended only when membrane formation remains unsuccessful even after spreading multiple bubbles over the aperture. Following this treatment, the formation of lipid plugs will be more frequent at first. In that case, break the plugs as described in the “Paint the membrane” section and continue dispensing bubbles until stable membranes form, indicating that the lipid concentration around the aperture is optimal.