BLM KIT

Elements Miniaturized Educational Kit (including an eONE Amplifier)

USER'S QUICK GUIDE

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This quick guide provides some brief information to successfully set up a complete Bilayer Lipid Membrane system using the BLM Kit.

The guide is divided in the following sections:

1) Instructions for software installation

2) BLM Kit content and setup

3) Instructions to properly perform a typical experiment:
    1. Electrodes chlorination
    2. BLM formation and protein insertion
    3. Teflon (or Polyimide) septum and BLM chamber's cleaning

4) Example data obtained from users with the BLM Kit

1) Instructions for software installation

1) Download EDR3 software from http://elements-ic.com/downloads/
2) Unzip the archive and start the installation following the instructions
3) Plug the eONE amplifier to any USB port of your computer and wait for hardware driver installation

2) BLM KIT content and setup

The kit is contained into a 16 x 10 x 5 cm Faraday cage and includes:

- The amplifier belonging to the eONE family
- one recording chamber composed by two Delrin-made cuvettes embedding the holes for the Ag/Cl electrode (one for each chamber).
- a set of laser-drilled Teflon-made septa embedding the micro-hole (50, 80, 110 µm sized) and/or a set of Polyimide septa embedding the micro-hole (100, 150, 200 µm sized).
- a paintbrush.
- Ag electrodes and electrodes holders to be connected to the eONE amplifier.

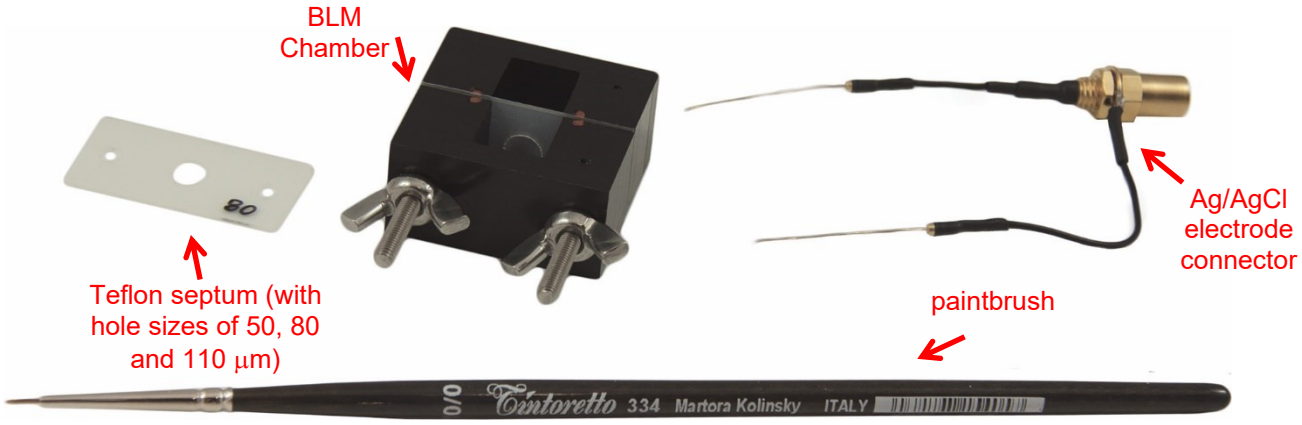


Figure 1: BLM EDU Kit content

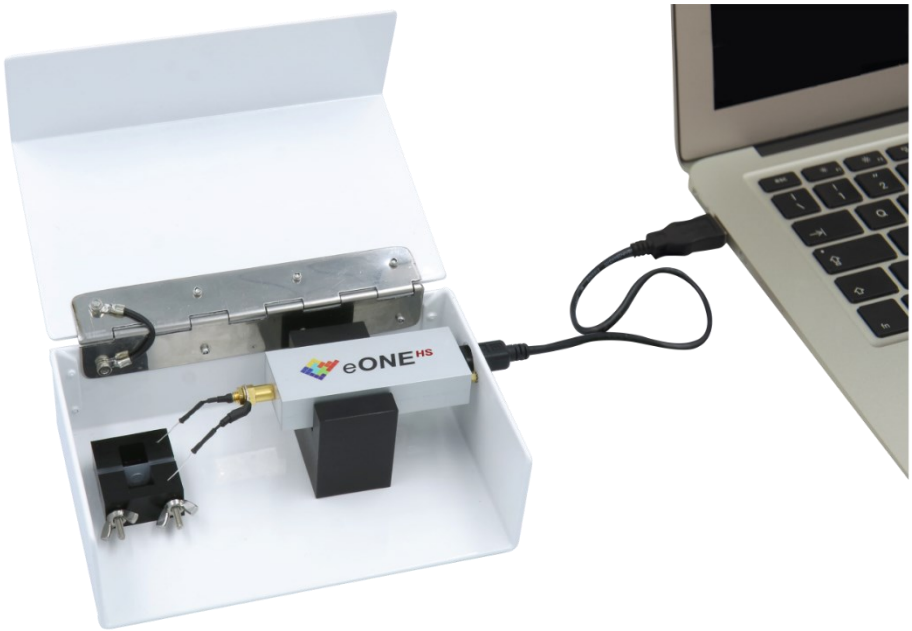


Figure 2: BLM Kit experimental setup example

The typical connection scheme is shown in the figure below:

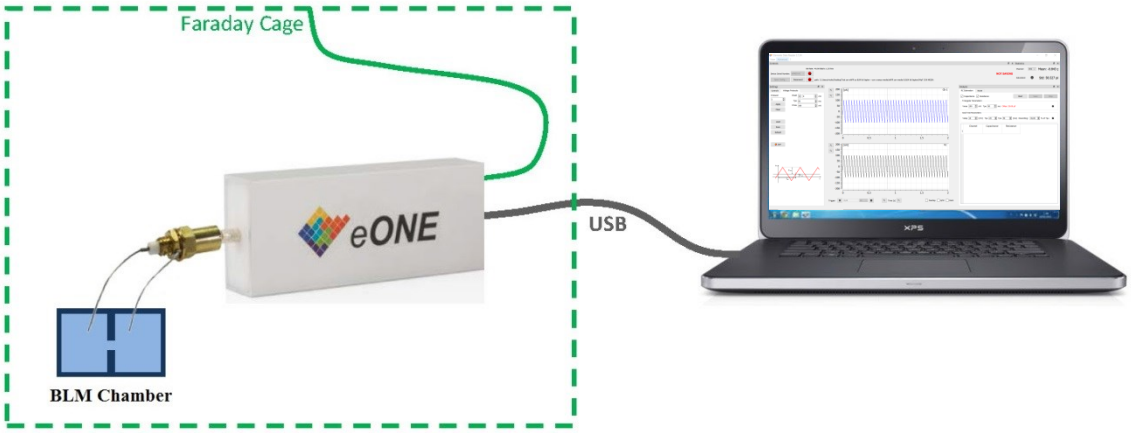


Figure 3: BLM Kit connections

Warning:

Do not short-circuit the electrodes with the Faraday cage!

3) Instructions to get started with a typical experiment

3.1) Instructions for chlorinating the Ag/AgCl electrodes

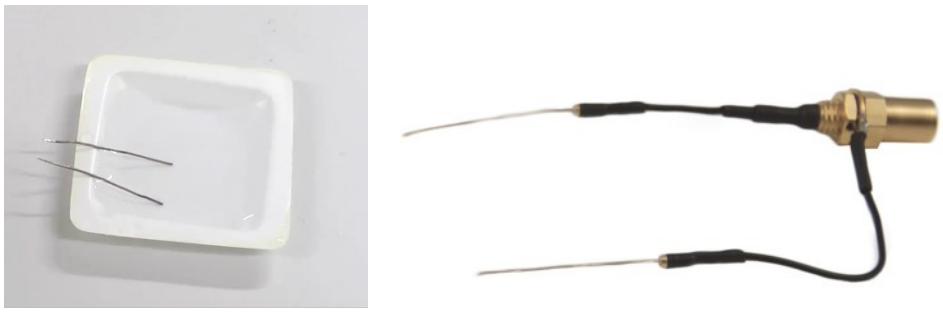


Figure 4: Ag/AgCl electrodes connector

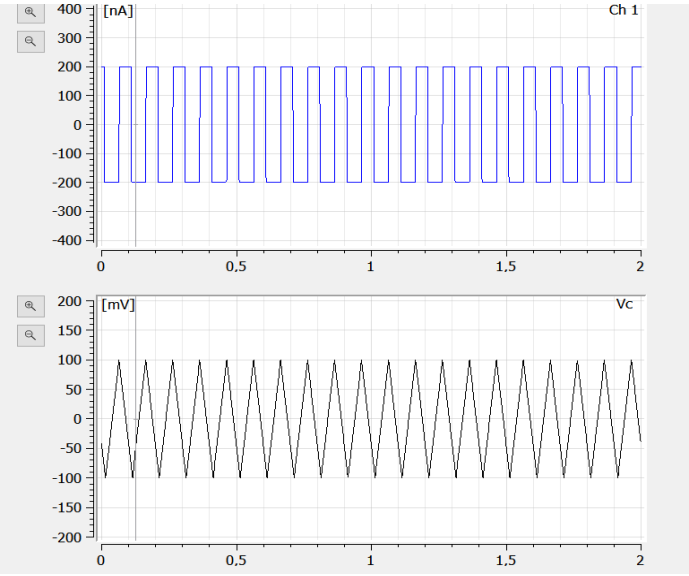
1) Before chlorinating, clean the Ag wires:
   - If it is the first usage, simply clean the wires with ethanol and rinse with double-distilled water.
   - If the wires were previously chlorinated, remove the old Ag/AgCl coating by abrading the surface with an extra fine grit sandpaper; then rinse with ethanol and double-distilled water.

2) Chlorinate the silver wires:
   - The simplest method to chlorinate the silver wires is to immerse them into pure bleach until they become light grey coloured (typically 15 to 30 minutes are enough). The commercial bleach purchased in any supermarket is fine for this purpose.
   - An alternative way that requires a little more effort but yields a deeper and more uniform chlorination is the electroplating method. Chlorinating a silver wire using the electroplating is achieved by making it positive relatively to a solution containing NaCl (0.9%) or KCl (1 M) and passing a current at a rate of approximately 1 mA/cm^2 for about 1 minute (or until the wire is adequately plated and becomes gray coloured). For example, to chlorinate a 1 cm length of a 0.5 mm Ag wire (that is the diameter of the wires used in the kit) requires 0.15 mA of current.
     While plating, occasionally reverse the polarity for several seconds; this helps to deepen the chloride coating and yields a more stable electrode.

The chlorination must be performed every time the current baseline is not stable overtime. It is recommended to perform a chlorination every 5 hours of continuous experiments.

3.2) Instructions for Bilayer Lipid Membrane formation (by painting method)

1) Prepare the lipid solution (here we suggest a protocol for DPhPC lipids): dissolve the lipids at 10 mg/ml in 95% n-decane (or n-octane) and 5% butanol.
2) Using the paintbrush, pretreat the micro-hole of the Teflon (or Polyimide) by painting a solution made of 10% hexadecane in pentane (or hexane) to increase lipids affinity to the septum in the area surrounding the micro-hole.
   (Although the pre-treatment provides a beneficial effect, it is not essential and sometimes it may also increase the probability to form lipid plugs)
3) Wait 5 minutes for the solvent evaporation.
4) Launch the EDR3 software.
5) Set the 20nA range.
6) Apply a triangular wave input signal (protocol 1).
7) Fill the BLM chambers with buffer solution. The top level of the solution must be above the hole of the septum (usually 700 μl are enough);
8) Apply the triangular wave input signal (protocol 1) and check the proper filling of the hole. A full-scale square wave (due to amplifier saturation) should be seen:



When the saturation of the current signal is not achieved, it means that the hole is not totally wet. In the first usage of a new Teflon (or Polyimide) septum, the hole can be occluded by air bubbles resulting in a reduced amplitude square wave like the one that can be seen when BLM is formed; if this happens, remove the buffer solution and fill the BLM chamber again by pushing the solution against the hole of the septum using the pipette. Another method is to give some mechanical shocks to the chamber in order to help the hole wetting.

9) Compensate electrode offsets by clicking on the “Voltage offset compensation” button. Depending on the EDR version this button can be found either in the settings panel, or as a miniature version next to the oscilloscope (see the red square in the

figures below). The mean current will reach a value close to 0 nA. At this stage, disable the Digital Compensation tool before proceeding to any other step. If not disabled, everything is measured afterwards is meaningless. This action is required to set the current at 0pA when a conductive path is present between the two electrodes, without any bilayer formed or any occlusion in the Teflon (or Polyimide) septum.

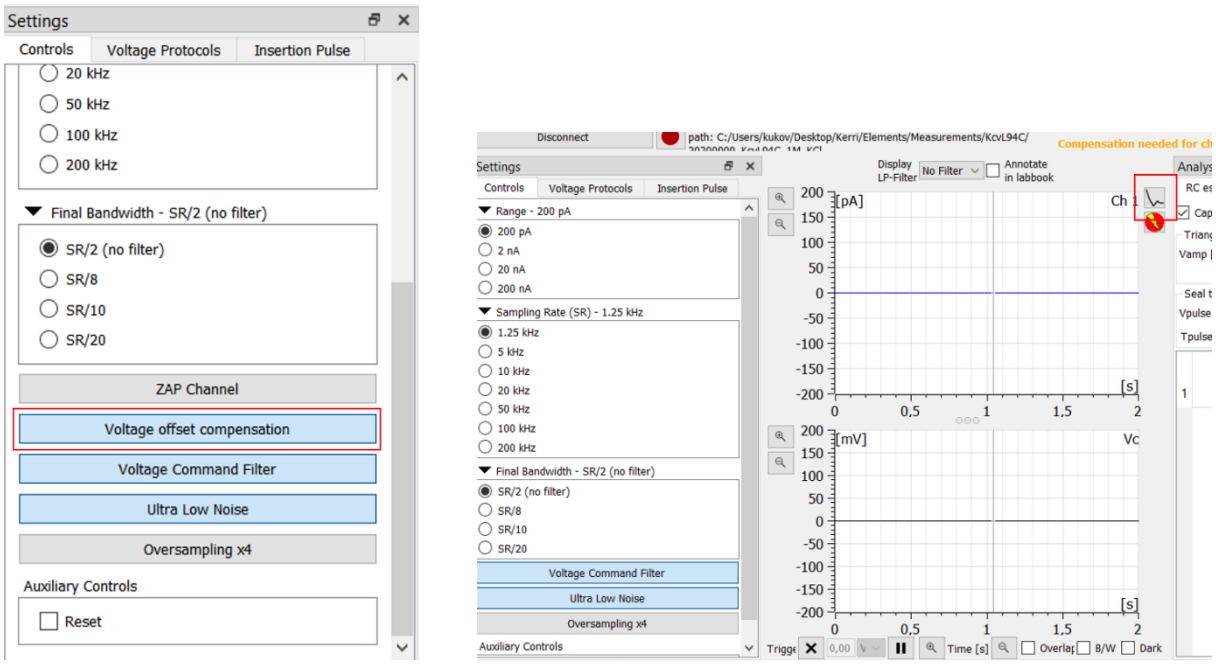
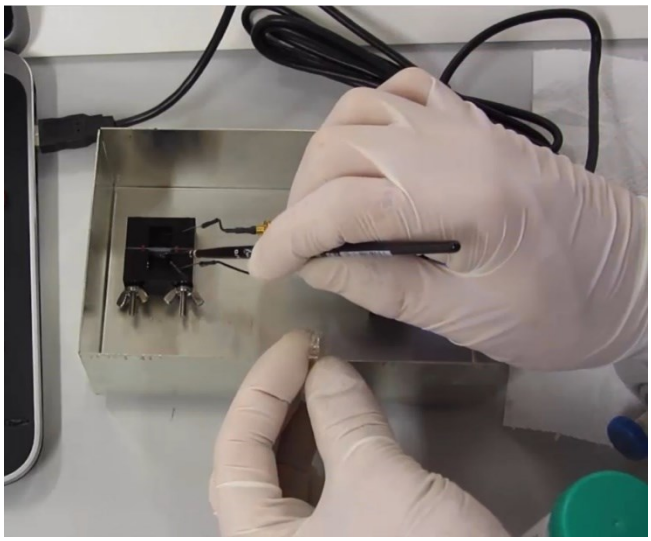


Figure 5: Digital offset compensation

10) Start to gently paint the lipid solution on the hole in the Teflon (or Polyimide) septum using the paintbrush: dip the paintbrush in the lipid containing solution and shake it to remove the excess of solution that may cause a lipid plug formation.



11) *Figure 6: Painting method*

12) To monitor membrane formation use the triangular wave input signal:

- When no membrane is present, a full-scale square wave due to amplifier saturation (in case of high-conductive buffer solution used) or a triangular current signal (in case of low-conductive buffer solution and small holes in Teflon septum is used) can be seen.
- when the membrane is formed a time-dependent square wave current signal can be seen in the display window (its amplitude increases proportionally to the BLM capacitance).

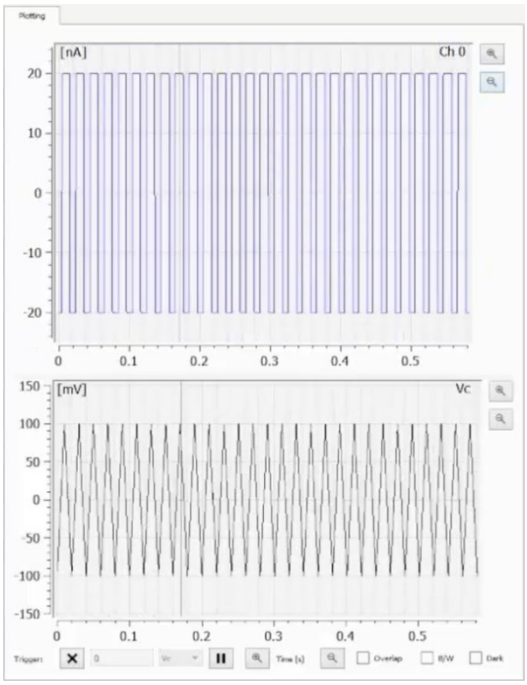


Figure 7: No-BLM situation current signal

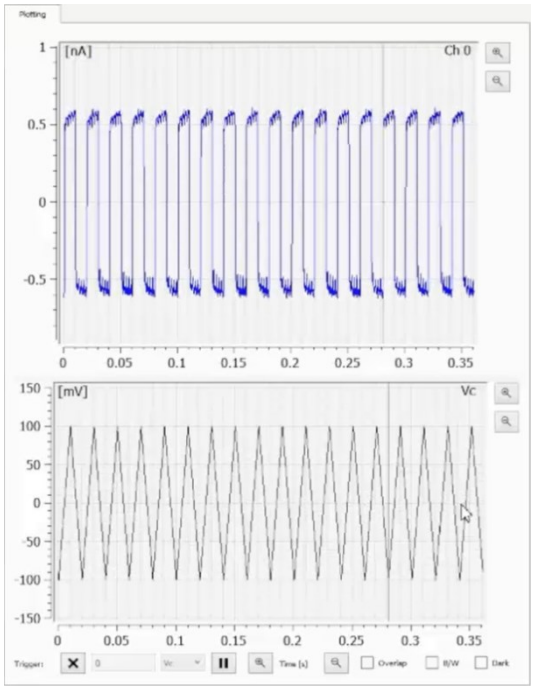


Figure 8: Formed BLM situation current signal

13) If no membrane forms, keep on adding small quantities of lipids to the septum following these steps:
    - Clean the paintbrush by dipping it in an ethanol containing-tube;
    - Accurately dry the paintbrush using a wipe;
    - Dip the paintbrush in the lipids solution and remove the excess by shaking
    - Add other lipid solution to the hole of the Teflon (or Polyimide) septum by painting;
    - Repeat the procedure until the membrane is formed.
14) Once the membrane is formed, apply the desired input signal (see the different voltage protocols in the EDR guide) and add the ion channel, ionophore etc.
    For instance, α-hemolysin toxin (aHL - H9395 SIGMA-ALDRICH product code) in double distilled water (or buffer) solution can be used. It could be useful to prepare aliquots (e.g., 10 μg/ml concentrated aliquots and add small amounts (0,1-0,5 μl) to the solution contained in the chambers, to have a final concentration in the chambers of some ng/ml).

Tips to make successful experiments:

- Use clean double distilled or Milli-Q water and fresh filtered (0.2 μm) electrolyte solution.

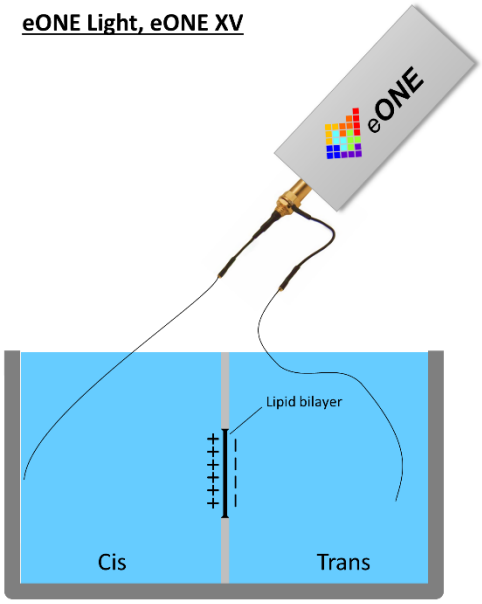
- To avoid "lipid plugs" the lipid solution can be diluted more (like 5 mg/ml DPhPC in n-nonane or n-decane).

- After monitoring the BLM formation using the triangular wave protocol, set a constant voltage (for instance +100 mV). If you can't see any insertion, try to add some more protein in solution; keep in mind that the most crucial point is the quality of the BLM. Sometimes, especially at the beginning of the experiments, it could be useful to break and form the lipid membrane again to promote BLM thinning and protein insertion and avoid "lipid plug".
  A good BLM, for instance, should have a capacitance of about 25pF (or larger) if you are using Teflon membranes with 80μm hole; and a capacitance of at least 35-40 pF if you are using holes of 110μm in diameter. All the typical capacitance values, experimentally obtained for BLM in the different holes, are reported in a table of our website (see the section “Septum & Properties): https://elements-ic.com/blm-kit/. If the capacitance value is smaller than those reported in the table for a long time (15-20 min) a “lipid plug” has probably formed; so, gently spread the lipids using the paintbrush without adding any lipid solution.

- To avoid lipid plugs it is also important to dry with a wipe (or an air flux) as better as possible the paintbrush before dipping it in the lipid containing solution.

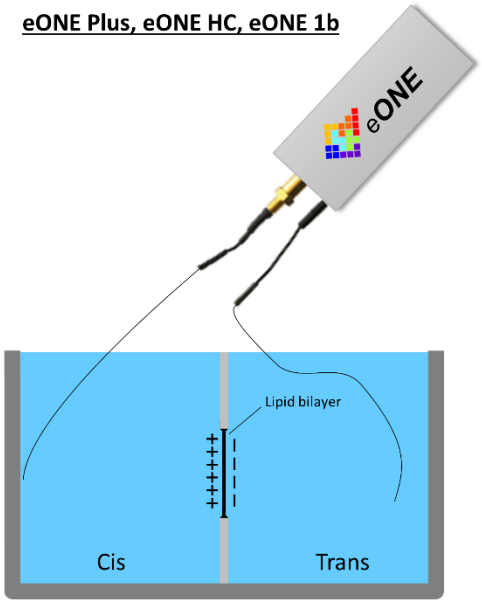
- Since the αHL protein is slightly negatively charged in buffer solution at pH 7, it could be convenient to add it in the cis chamber when a constant positive voltage is applied. The polarity of the membrane when a positive voltage is applied is shown in the figure below. The connection diagrams and the schemes of eONE amplifiers series circuitry are illustrated in the dedicated How-To guide.

eONE Light, eONE XV



If V command is +100 mV

eONE Plus, eONE HC, eONE 1b



If V command is +100 mV

3.3) Instructions for Teflon (or Polyimide) septum and BLM chamber's cleaning

Warning:

Do not use Acetone to clean Teflon septum

- The safest way is to clean the Teflon (or Polyimide) septum by using ethanol to remove lipids; then simply rinse the septum with double-distilled water.

- Another procedure is to use trisodium phosphate (TSP, Na_3PO_4) solution (40-50 mM), which is a stronger cleaner than ethanol.

  In this case you can use the following procedure to clean the supports:

  1) Rinse the chambers with double-distilled water to remove the water-soluble components
  2) Remove the Teflon (or Polyimide) support from the BLM chamber
  3) Clean the hole in the septum using the trisodium phosphate (TSP) solution;
  4) Rinse with dilute HCl solution (0.1% by volume) to remove any residual TPS;
  5) Rinse with double-distilled water to remove any remaining HCl;
  6) Gently dry the septum using a wipe or a low air jet.

Warning:

Since the Teflon septum is very fragile, be careful to not apply a high pressure on them while rinsing or drying.

4) Example data obtained from users with the BLM Kit

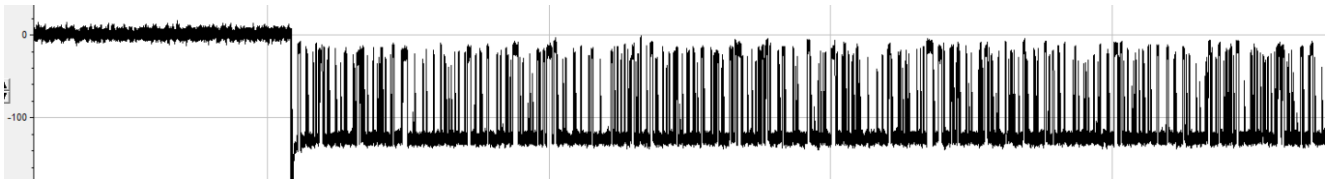


Figure 9: Translocations of **PEG 1500** through a **α-hemolysin** nanopore.
Buffer solution: 3M KCl. -40mV transmembrane voltage applied. Data were recorded at 10 KHz final bandwidth (20 KHz sampling rate without filter).
(Data from A. Oukhaled, LAMBE UMR 8587 CNRS, Évry and Cergy University, Cergy-Pontoise 95011 cedex, France)

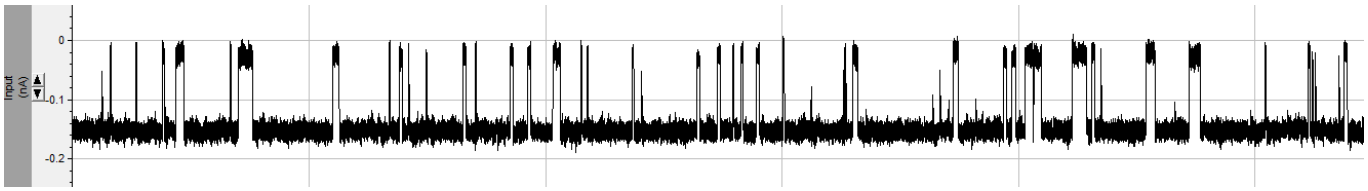


Figure 10: Current pulses of **PEG 4000** through single **α-hemolysin** nanopore.
Buffer solution: 4M KCl. -40 mV transmembrane voltage applied. Data recorded at 10 KHz final bandwidth (20 KHz sampling rate without filter).

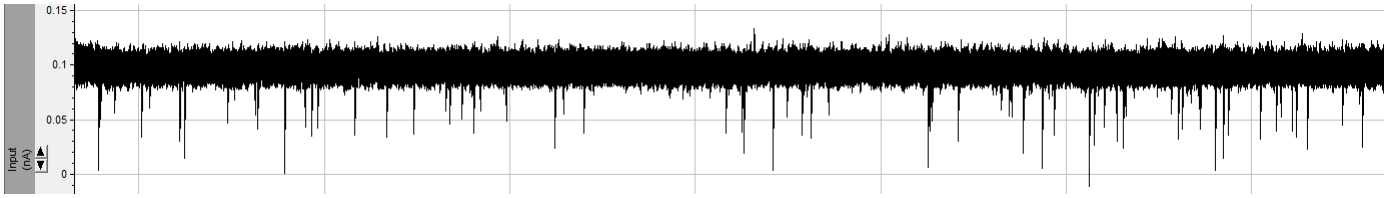


Figure 11: Current pulses of **Poly(dC)$_{50}$** through single **α-hemolysin** nanopore.
Buffer solution: 1 M KCl. 100 mV transmembrane voltage applied. Data recorded at 5 KHz final bandwidth (10 KHz sampling rate without filter).

(Data from Prof. Zhi-Yong Wu's group, Research Center for Analytical Sciences, College of Sciences, Northeastern University, Shenyang, Liaoning 110819, China)

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