Suspended bilayer flow cell instructions

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Single-channel current recordings are performed using planar lipid bilayers formed by the lipid monolayer folding technique, as previously described (Montal & Mueller, 1972). The *cis* and *trans* compartments of the chamber (Nanopore Solutions) are separated by a 25 or 50-µm-thick Teflon film (Goodfellow Cambridge Ltd., Huntingdon, UK, cat. Nº. FP301200 - 25µm film or cat. Nº. FP341050 – 50µm film) containing an aperture of around 120µm in diameter.

1. Assemble the flow cell by applying a thin layer of vacuum grease to the inner faces of the half-cells. Wipe away any excess grease.
2. Position the Teflon film, so that the aperture is in the center of the holes in the middle of the flow cell.
3. Tighten the flow cell using an allen key and the bolts provided.
4. Seal the openings at the base of the flow cell using parafilm.
5. The aperture in the Teflon film is pretreated with 10% v/v hexadecane in *n*-pentane. Store this solution at -20°C. Ensure that it is fully thawed and mixed prior to use. Apply the solution using a Pasteur pipette. Insert the small end of the Pasteur pipette into the solution and allow capillary action to draw solution into the pipette. Carefully place the end of the pipette over the aperture in the Teflon film and tap the wide end of the pipette to add the solution to the aperture. This must be performed on both sides of the aperture.
6. The flow cell should then be left for about 3 minutes for the *n*-pentane to evaporate.
7. Setup your Ag/AgCl electrodes
8. At this stage it is a good idea to nominate (or better still, to label) the halves of your flow cell. The *cis* side of the flow cell should contain the

ground electrode. The *trans* side of the flow cell will therefore contain the working electrode.

9. Set up your software to record in "Whole Cell" mode (a positive voltage means that the working electrode is at a positive potential and the ground electrode is at 0 V).
10. Both compartments of the flow cell are then filled with electrolyte solution containing *e.g.* 2 M KCl and 10 mM HEPES (pH 8.0). Each chamber holds approximately 1.4ml.
11. Position the electrodes, one in each half of the flow cell.
12. Auto-zero your system.
13. Apply a triangular wave using the "pulse generator" within your software. +5mV to -5mV over 10ms. Loop this program, or cycle for as many cycles as you can. The output of this should be a rectangular signal. A good pretreatment results in a rectangular signal from between +11 to +17 pA and -11 to -17pA. Ideally 13-15pA is the desired current.
14. 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL, cat. Nº. 850356P) in *n*-pentane (10 mg mL^{-1}) is then added to both compartments. This solution must be stored in small aliquots (*e. g.* 200 µl) in glass vials at -20 ºC. This is applied using a Pasteur pipette. Insert the tip into the stock solution and allow capillary action to draw it into the pipette. Touch the tip of the pipette to the buffer solution in the *cis* side of the flow cell. Repeat this procedure for the *trans* side. Allow a few minutes for the *n*-pentane to evaporate.
15. After evaporation of *n*-pentane, the lipid bilayer is formed by lowering and raising the electrolyte level in one or both sides of the flow cell. The level needs to be lowered below the level of the hole in the center of the flow cell.
16. Repeat the triangular wave from step 11. A well formed bilayer should give you a current reading of between 120-150pA. If the bilayer is too thick then the current will be reduced. If it is too thin, the bilayer will be be very fragile.
17. If the bilayer is not of a desired thickness, break it by increasing the voltage to a very high level (e.g. 800mV). Repeat steps 13 and 14.

Note that continuously repeating this process will eventually remove the pretreatment applied in step 5.

18. Apply the potential that you are planning to work at (usually 80-100mV).
19. α-Hemolysin (wild-type) protein (*e.g.* Sigma, Saint Louis, MO, cat. Nº. H9395) is then added to the *cis* compartment in its monomeric form. A 0.5mg/ml stock solution of the commercial protein monomer is made in distilled water. Store 5µl-aliquots at -20ºC. Dilute the stock 100x in distilled water or 20 mM HEPES buffer, pH 7.5. Add 0.75-1µl of the previous solution on the *cis* chamber, in order to get a single pore. In this way, the protein nanopore presents its mushroom cap facing the *cis* compartment, while the β-barrel is spanning the lipid bilayer, facing the *trans* compartment of the chamber.
20. Repeat the triangular wave to check that the membrane is still intact.
21. Again, apply the voltage that you intend to work at and wait for a pore to form. The open pore current values are described in the literature. Briefly, under an applied potential of *e.g.* +100mV, the single pore current will be around 200pA. If two pores insert into the bilayer, the current will be doubled. Since α-hemolysin partially rectifies the ionic current at negative voltages (Aksimentiev & Schulten, 2005) lower current values are expected. Thus, under an applied potential of -100mV, the single pore current will be around -160pA. If the current is symmetric, then there is a leak in the bilayer and it must be reformed.
22. After a single protein nanopore has formed in the lipid bilayer a potential difference is applied. Positive potentials of +40, +60, +80, and +100 mV were applied across the lipid bilayer with Ag/AgCl electrodes. The *cis* compartment was grounded so that a positive potential indicates a higher potential in the *trans* compartment, meaning that positive charges are moving from the *trans* to *cis* side of the lipid bilayer.
23. After each usage, electrodes must be rinsed with distilled water and air dried. To clean the chamber, rinse it with (tap) water, ethanol, and

distilled water and leave to air dry. There is no need to disassemble the chamber – the Teflon film can be left in the assembled chamber.

Notes:

Electrodes can be reconditioned by scraping the silver part of the electrode with a blade, or rubbing with sandpaper or wet and dry paper. Clean away any excess material and leave the electrode in bleach overnight. Wash with distilled water and leave to dry).

We recommend connecting the earth lead from your Faraday cage to the earth electrode.

To shield from external electrical noise we recommend the use of a faraday cage (e.g. Clutch™ from Nanopore Solutions), inside which the bilayer recording amplifying headstage, chamber, and electrodes are all enclosed.

For current measurements we use a patch clamp amplifier (EPC8, HEKA Elektronik, Lambrecht/Pfalz, Germany). Data is acquired using PatchMaster software (HEKA Elektronik, Lambrecht/Pfalz, Germany). Recordings are collected using a 10 kHz low pass Bessel filter at a sampling frequency of 30 kHz using a computer equipped with a with a LIH 8+8 A/D converter (HEKA Elektronik, Lambrecht/Pfalz, Germany). All measurements are performed at room temperature.

References

Montal, M.; Mueller, P., Formation of Bimolecular Membranes from Lipid Monolayers and a Study of Their Electrical Properties. *P Natl Acad Sci USA* **1972,** *69* (12), 3561-3566.

Aksimentiev, A.; Schulten, K., Imaging α-Hemolysin with Molecular Dynamics: Ionic Conductance, Osmotic Permeability, and the Electrostatic Potential Map. *Biophysical Journal* **2005,** *88* (6), 3745-3761.