Bilayer Protocol for Single Channels

1. **Turn on all instruments**. Let them warm up for a few minutes prior to use.
2. **Paint the membrane**:
   a. Clean cell and cup with EtOH then dry with N2. Make sure both are completely dry, especially the area around the glass portion of the cell.
   b. Get lipid from left most freezer (in door in screw-top container) and warm briefly with hands
   c. Use biggest (blue) brush and dip into lipid (ONLY the bristles). Shake to remove excess lipid then dab lipid onto hole (front first, then back). Can check coverage by placing finger over the top of the cup and looking for lipid bubbling through.
   d. Dry cup with open side up for ~10 minutes. Wash brush with Folch 4x (dip in ONLY the bristles then dry on filter paper). Put brush in rack when clean (ONLY clean brushes go in the rack).
3. **Initial apparatus setup**:
   a. Insert electrodes (1 male, 1 female; use pellet electrodes). Male goes into headstage, female goes into wire. Use tweezers to remove electrodes from storage box. At this point make sure the plastic cover is around the tip of the headstage.
   b. Fill salt cups (with 1 mL of 3M KCl) and place into holders.
   c. Lower electrodes into salt solution (use knob to loosen headstage in order to reposition it). Position male electrode first, then female; pellet portion of electrodes should be submerged in salt solution.
4. **Setup the cell**:
   a. Place cup in cell (open side up) and position such that the hole is centered when looking through the glass.
   b. Tighten the screw to hold the cup in place - want it to be tight so no buffer leaks from one compartment to the other.
   c. Add 1 mL buffer to each compartment (front and back). Usually 10 mM Succinate pH 6.6, 100 mM KCl, 1 mM EDTA buffer is used, but you can use something different. Add a stir bar to the front compartment.
   d. Slide cell into apparatus (on top of white plastic).
   e. Setup the salt bridges
      i. Remove salt bridges from buffer storage, dry quickly with kimwipe, then place into salt cups. Back salt bridge connects male electrode salt cup to back compartment of cell. Front salt bridge connects female electrode salt cup to front compartment of cell - but through the small hole to the left of the font compartment. It's important to use KCl salt bridges if you're using KCl in the salt cups.
5. **Setup the instruments**:
   a. Use function generator (top machine) to get the triangle wave output. Hit recall button then 1 (for triangle wave) then enter. Make sure output light is on. Now triangle wave function is being produced.
   b. Turn on external command switch on amplifier. Set gain to 100 and change channel 1 setting to 5V (normally set to 100 mV).
   c. Open Clampex. Click on active signal button and set filter to bypass.

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6. **Form the membrane**:
   a. Center the function around 0, use pipette offset knob to do this. Some fluctuation is inevitable, but look at display screen to try and get a symmetric function.
   b. Paint the membrane (dip clean blue brush in lipid, shake off excess) by moving brush from bottom to top of the hole. When membrane has formed the vertical lines on the screen will be replaced with small rectangles.
   c. Pop the membrane (remove back salt bridge, place finger over top of cup, then replace salt bridge). Do this at least once, maybe more before getting to your final membrane. When the membrane has popped you'll see the vertical lines on the display screen.
   d. Bubble the membrane back by using a small bubble tool and sliding a bubble over the hole. The first pass of the bubble should restore the membrane (check display screen for small rectangles). Each consecutive pass should thin the membrane (which you'll see as an increased vertical dimension of the rectangles). The top of the rectangles should be flat - if not it means you have a hole in your membrane and should pop and re-bubble. Membrane is close to 'ready' when vertical dimension of rectangles goes through 2 X axis scale lines (though this can vary depending on the size of the hole in the cup and the particular cup you're using).
   e. When membrane looks good, start preparing sample. Dilute pre-assembled PA to ng/mL range (usually in pH 9 buffer because high pH keeps PA soluble).
7. **Recording Data**:
   a. Get 1 μL of sample in pipette (can use more than 1 μL but best to start on the low end).
   b. Check that stir bar works.
   c. Bubble membrane 1-2 more times, just to make sure it's as thin as you can get it. Re-zero using the pipette offset knob if necessary.
   d. Turn off the external command feature on the amplifier.
   e. Use the active signal button to change the filter setting to 200 Hz. Sample rate (on left side of screen) should be set to 400 Hz. Filter should be set to no more than 1/2 of sample rate.
   f. Set voltage (left side of screen) to -20 mV.
   g. Screw down lid of housing.
   h. As fast as you can do the following:
      i. Hit record button (red circle)
      ii. Add protein to the front compartment
      iii. Close door and screw down.
      iv. Turn on stir bar (briefly)
   i. Wait for channels to insert. If you don't see any channels in ~3 minutes, add more protein (either by volume or make a new dilution that is more concentrated).
   j. Data is saved as yearmonthday3digitnumber.abf; so an example file would be '08501000.abf'. It's important to write down the numbers you use so you can reopen them in Clampfit to analyze the data.
   k. <u>You can only use the membrane a certain number of times before you need to make a new one.</u> To do that, stop recording data and remove the cell from the system. Clean it out (dump liquid, remove stir bar, add 0.5 M NaOH and rub with cotton tipped stick,

then rinse with DI water, then EtOH, then dry with N2) and then reform the membrane just like during the setup.

8. **Cleanup**:
   a. Turn off ClampEx then turn off all the equipment.
   b. Remove salt bridges and put them away in buffer conical.
   c. Remove cell from holder and clean it thoroughly. Also clean cup thoroughly, including using NaOH to hydrolyze the lipid.
   d. Remove electrodes, wash salt off (GENTLY) with DI water then blot dry with kimwipes, then place in storage box.
   e. Dump out salt cups then rinse with DI water.
   f. Use a damp kimwipe to clean any salt off the headstage.
   g. Clean all brushes used with Folch then put away in rack.
   h. Put lipid away in the freezer.

Helpful Tips:

Make sure to ground yourself before you touch the setup after you start recording (try touching the door frame or the computer rack).

Place dirty brushes on a paper towel, place clean brushes in the rack.

Place clean cups with the open side down on a paper towel.