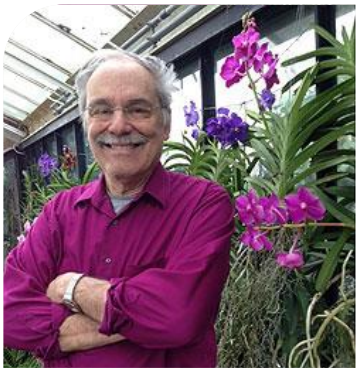
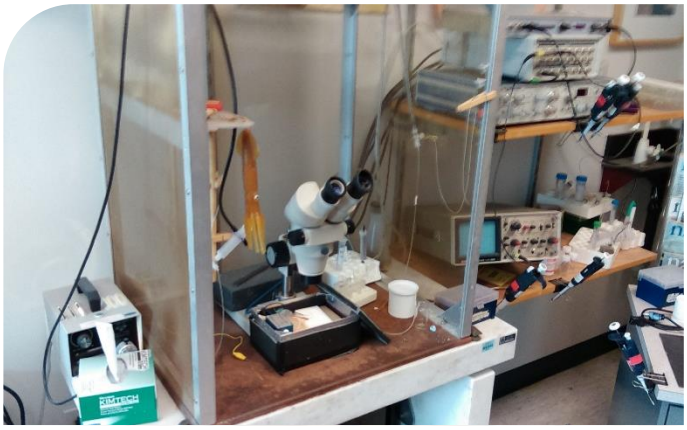
Getting going with the Orbit mini planar bilayer system

Dr. Chris Miller, Brandeis University

A narrative:



"After 40 years of using home-built planar lipid bilayers for recording ion channels from membrane fragments or purified protein preps, I recently purchased the Orbit mini system. This was motivated by my own curiosity regarding the noise-improvement claims made by Nanion, about which I was skeptical, and also by the compactness and mobility of the Orbit mini. Moreover, with more labs now functionally characterizing purified ion channel proteins in planar bilayers, the standardization offered by this instrument seemed a real advantage to the field in lowering barriers to new users of the technique, barriers that can seem quite daunting in setting up from scratch a home-built system. The purpose of this narrative is to compare my long experience with home-built bilayers with our first 2-3 months of adapting the Orbit mini to our current application – a ~10 pS fluoride-selective ion channel that shows rare closing events on the 10-ms timescale. All the work here was done by Dr. Nick Last, a postdoctoral associate not previously trained in electrophysiology. Nick's experience provides an excellent model for a skilled biochemist undertaking planar bilayer channel recording for the first time.



I compare the Orbit mini to our current planar bilayer system, optimized over decades and in daily use here. This consists of a hand-milled plastic chamber consisting of a horizontal partition with a hand-fashioned hole of ~50 µm diameter on which bilayers are painted, separating "upper" and "lower" aqueous chambers of 0.7 mL and 0.2 mL volume, respectively. Partitions are cut from overhead transparency film (80 µm thickness), are cleaned after use and stored in ethanol, and can be re-used literally for years. The chambers are connected through salt bridges to a standard patch-recording setup (Axopatch 200-series or Warner 505). The chamber and headstage are mounted in a metal box with lid, with all surfaces coated in soundproofing material, on a vibration-damping table. Bilayers are 'painted' while viewing the partition under a stereomicroscope. For highest-quality recordings, I typically use bilayers of 30-40 pF capacitance. To record our 1-pA F^- channel, we set the low-pass filter corner-frequency at 200 Hz, a where we can clearly discern the ~1-10-ms closing events (rms noise is ~ 0.4 pA). In addition to the headstage voltage-noise amplified through the membrane capacitance, a major source of noise in our bilayers – and one irritating in its variability from bilayer to bilayer and day to day – is microphonic pickup of sound in the room; this is often difficult to filter out because it includes low-frequency components.

Contrary to my initial expectations, the Orbit mini has performed exceptionally well for our application, with vastly improved signal-to-noise characteristics over our best recordings on the home-built systems. This was a surprise to me, since I had imagined that whatever the improvement in electronic noise, the ~50 pF bilayers that form on the holes in the recording chip-partitions – slightly larger than those I typically use - would still suffer the same microphonic disturbance that limits our time-resolution. This was not the case. To record a 1 pA channel, we can now set the filter frequency to 625 Hz to achieve the same rms noise as above. This represents a 3-fold improvement in signal to noise, a really enormous advantage of the Orbit mini system. I do not understand why bilayers of similar size respond so differently to microphonic pickup; this may be because of the very tiny volume of the lower chamber in the Nanion instrument (~20 pL !).

The compactness of the Orbit mini makes for flexible and convenient placement. With a tiny footprint, it can sit on a table or desk, accompanied by only a laptop. The entire package of Faraday cage and rig-rack is eliminated. The

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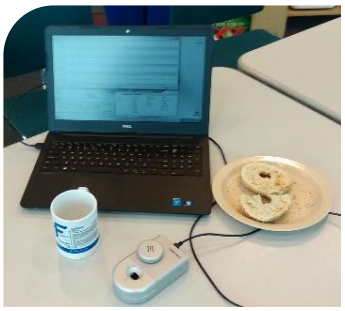
acquisition software is intuitive and robust, and the data can be stored in formats readable by the Axon analysis programs that we routinely use. Moreover, the four electrically independent holes in the mircro electrode chip array (MECA)-partition means that four bilayers can be monitored simultaneously, thus quadrupling the probability of catching a clean single channel to record. This represents a significant practical boost in experimental efficiency.
Our learning curve in taming the new Orbit mini was smooth on the whole, but we experienced a few bumps along the road that I list here – problems for which the Nanion technical support staff were very responsive and helpful."

Practical discoveries using the Orbit mini system:

1. Deterioration of chip-partitions. Nanion claims that each MECA chip-partition (cost ~$65) can be washed and re-used for about 3-5 days without deterioration. But for the first few weeks, we could form stable bilayers only on the first day after opening a new chip. We eventually tracked this down to our standard inclusion of 50µg/mL serum albumin in all our solutions (to suppress binding of added proteins such as antibodies to the chamber walls). We cannot so far wash this residue away, but if we simply omit this component, the chip lifetimes are as advertised.



2. Lipid solution for bilayer formation. We have always used 10 mg/mL phospholipid in n-decane for forming bilayers, but this does not work well on the MECA solid-state partitions. Instead, we find that n-nonane is an excellent lipid solvent in the new system – better than the n-octane recommended.

3. Mechanical delicacy of the partitions. In contrast to the tough plastic of our home-made partitions, which tolerate mechanical abuse in painting bilayers, the rather delicate MECA partitions require a gentle hand while applying lipid to the holes. The teflon film wand that Nanion supplies, when wielded with Nick's light touch, works well, while my own bubble-paintbrush method, so well suited to my shaky hands, too often breaks the partitions. However, a distinct advantage is that the teflon wand method can be easily accomplished 'blind,' without needing a microscope or illuminator to view the partition, a circumstance that further reduces the footprint of the system.

Some maneuvers often used in our home-built systems that cannot be done with the Orbit mini:

1. Constraints regarding Cl^-. Since the Ag-AgCl electrodes are built into the MECA chip-partitions, Cl^- must always be present (preferably at equal concentrations on both sides of the bilayer). It is not possible to use salt bridges to connect the electrodes to Cl^--free solutions. All our F^- channel recordings necessarily are made with 10 mM Cl^- added to both 200 mM NaF solutions – not a problem for us because the inertness of Cl^- for this channel was already established (in our home-built system). But this could be a serious problem for anyone studying anion selectivity properties.

2. Perfusion of trans chamber. The lower chamber cannot be perfused, and so, as with standard patch-recording, only the upper "bath" solution may be changed during an experiment. This precludes one of the strengths of planar bilayers – straightforward double-sided perfusion while recording a channel.

3. Application of solute gradients. Because of the small volume of the lower chamber and the way solution is introduced to the Orbit mini, both chambers must be initially filled with the same aqueous solution. Only after bilayer formation can a solute gradient be applied, and only by changing the upper solution, an easily accomplished maneuver. If the bilayer breaks, any gradient will quickly be dissipated.

Publications:

Molecular determinants of permeation in a fluoride-specific ion channel. Last N.B., Sun S., Pham M.C., Miller C. (2017) eLife 6:e31259

Mechanistic signs of double-barreled structure in a fluoride ion channel. Last N.B., Kolmakova-Partensky L., Shane T., Miller C. (2016) eLife 5:e1876