

# Flip the Tip: An Automated, High Quality, Cost-Effective Patch Clamp Screen

**Albrecht Lepple-Wienhues and Klaus Ferlinz**

*Flyion GmbH, Tübingen, Germany and Department of Physiology, University of Tübingen, Tübingen, Germany*

**Achim Seeger and Arvid Schäfer**

*Physiology, University of Tübingen, Tübingen, Germany*

---

**The race for creating an automated patch clamp has begun. Here, we present a novel technology to produce true gigaseals and whole cell preparations at a high rate. Suspended cells are flushed toward the tip of glass micropipettes. Seal, whole-cell break-in, and pipette/liquid handling are fully automated. Extremely stable seals and access resistance guarantee high recording quality. Data obtained from different cell types sealed inside pipettes show long-term stability, voltage clamp and seal quality, as well as block by compounds in the pM range. A flexible array of independent electrode positions minimizes consumables consumption at maximal throughput. Pulled micropipettes guarantee a proven gigaseal substrate with ultraclean and smooth surface at low cost.**

---

**Keywords** Gigaseal, High Throughput Electrophysiology, Ion Channel, Patch Clamp Automation, Whole Cell Recording

Since the completion of the human genome project, academic and pharmaceutical research has focused on assembly and function of proteins including ion channels. Ion channels are involved in rapid signaling, electrical excitability, and fluid transport throughout the body. Therefore, ion channels are becoming an increasingly important drug target. Ion channel blockers and openers represent potential therapeutic drugs for a variety of diseases. Furthermore, certain cardiac ion channels are responsible for major toxicology problems like the long QT syndrome, requiring ion channel screening in an early stage of the drug discovery process for pharmaceutical safety (Numann and

Negulescu 2001). However, measuring ion channel function has been a painstaking, time-consuming, and expensive process. Indirect methods, including radioactive tracer flux and voltage dye fluorescence assays, have been unreliable and do not provide the essential means to study these nanoscale pores: voltage control and close monitoring of the electrochemical driving force.

The most advanced direct method to study ionic currents through biological channels is the patch clamp method. It provides complete, microsecond scale control over the electrical and chemical environment of the channel pore. However, prodding a cell with the tip of a micropipette on an antivibration table underneath the microscope requires considerable skills, expensive tools, and plenty of time.

Patch clamp automation has faced three major obstacles:

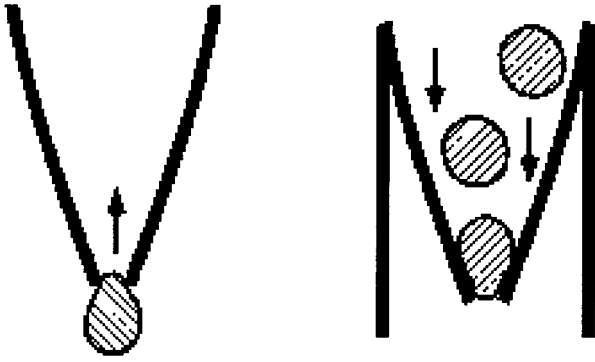
- Prepare an ultraclean surface on a suitable substrate to glue to cell membranes, obtaining the required gigaseals for measuring tiny currents
- Position single mammalian cells in a micron scale without microscope and micromanipulator
- Handle complex fluidic and electronic procedures to automatically perform the complicated steps involved in a whole-cell patch clamp experiment.

In recent years, many approaches have been used to rid the patch clamp of microscope, micromanipulator, and scientific operators (Fertig et al. 2002; Sigworth and Klemic 2002; Klemic et al. 2002). Several examples for patch clamp on a chip, i.e., “planar opening technologies,” are described elsewhere in this issue.

We have taken a different approach to overcome the obstacles for automated patch clamp. Years of experience in the patch clamp field have shown that glass is a low cost, proven gigaseal substrate (Sakmann and Neher, 1995). Glass melting produces ultraclean, smooth surfaces. Pulling glass capillaries to small

---

Received 1 January 2002; accepted 12 April 2002.  
All methods, procedures, data, and apparatus described or shown are property of Flyion GmbH (patents pending).  
Address correspondence to Albrecht Lepple-Wienhues, Flyion GmbH, Waldhäuserstr. 64, D-72076, Tübingen, Germany. E-mail: info@flyion.com



**FIG. 1.** Conventional patch clamping requires meticulous micromanipulation and microscopic control. The resulting preparation is mechanically fragile (left). In contrast, flushing suspended cells into glass pipette tips automatically achieves extremely stable gigaseals at a high rate (left). Microscope and micromanipulator are no longer required.

tips is a reliable, cost effective, and simple method to create holes in a micrometer scale.

Therefore, we studied whether it was possible to produce gigaseals and whole-cell preparations inside a glass micropipette by simply filling the pipette with cell suspension and flushing cells toward the pipette tip (Figure 1).

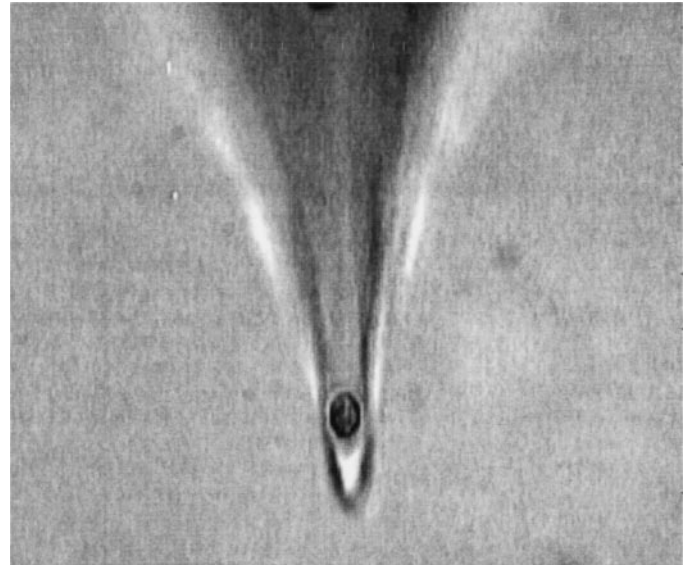
### GIGASEALS INSIDE A PIPETTE TIP

Using a variety of different cell types expressing various ion channels, we tested whether gigaseals could be routinely produced with this new method. Surprisingly, we were successful in obtaining gigaseals ( $R > 1 \text{ G}\Omega$ ) at a high rate using human Chinese hamster ovary (CHO) cells, Chinese hamster lung cells, Jurkat T lymphoma cells, human embryonic kidney cells, mouse erythroleukemia cells, and others.

Since most patch clamp experts assumed that the rim of the pipette was important for seal quality, we studied where the seal was forming with this method and whether the seal resistance/quality was comparable to the conventional technique. The micrograph in Figure 2 shows that the seal formed between the outer circumference of the rounded cell and the smooth inner surface of the glass pipette.

Surprisingly, the seal resistances were as high as with conventional patch clamp. In a typical experimental run, the seal rate with CHO cells was 82% and the seal resistance  $9.3 \pm 1.7 \text{ G}\Omega$  (mean  $\pm$  SEM,  $n = 378$ ). With Chinese hamster lung cells, the respective figures were 75%,  $11.0 \pm 1.0$  ( $n = 281$ ). The threshold for a successful seal was set at  $1 \text{ G}\Omega$ .

Most notably, for these high rate/high quality seals, only minimal apparatus equipment was needed. The pipette tips were originally inserted into a piece of tubing filled with intracellular saline that contained an Ag/AgCl electrode. Cells suspended in extracellular saline following application of trypsin/EDTA were



**FIG. 2.** Micrograph using a  $60\times$  Zeiss DIC objective showing a Jurkat T lymphocyte sealed inside a glass capillary.

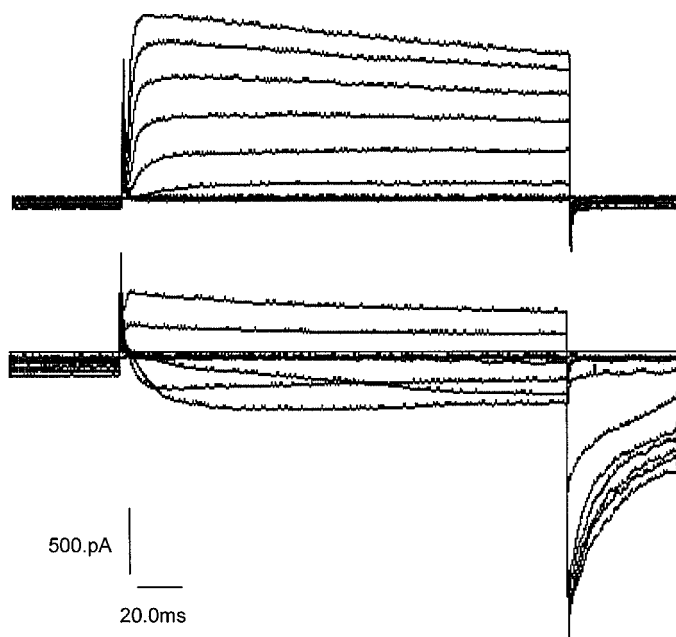
filled into the glass micropipette. Suction applied toward the tip resulted in seal formation within 10–90 s.

The gigaseals inside the pipette were extremely durable. Tapping against the pipette, flushing the pipette with saline, or even moving the pipette/tubing assembly from one holder to another did not break the seal. Therefore, the method was ideally suited to design a robot that forms seals, establishes whole-cell access, and adds compounds automatically.

### WHOLE-CELL PREPARATIONS

For most screening scenarios the whole-cell configuration of the patch clamp method is usually preferable since currents are larger and the channel proteins behave more physiologically than in other configurations (Numann and Negulescu 2001). A whole-cell preparation demands physical or chemical disruption of the cell membrane only on one side of the seal. This is done in order to minimize electrical access resistance and to optimize diffusional access to the remaining, intact cell membrane (Penner 1995).

Surprisingly, a true whole-cell configuration was achieved simply by application of suction pulses to the cell sealed inside a glass micropipette. Suction toward the pipette tip disrupted the membrane area facing the tip. Since only discrete constant suction pulses were needed instead of a feedback pressure control, automating seal and whole-cell formation was an easy task. A software routine monitored resistive and capacitive currents during a  $5 \text{ ms}/5 \text{ mV}$  test pulse and controlled the pressure line. Seals and whole cell access were routinely obtained without any human interference. Typically, 1–5 suction pulses were needed for disrupting the membrane surface located close to the pipette tip.



**FIG. 3.** The voltage gated  $K^+$  channel Kv1.3 in standard saline (upper) and symmetrical  $K^+$  (lower) using a Jurkat cell perforated with amphotericin. Voltage step responses from 60 mV holding were overlaid. Standard extracellular saline contained (mM) 145 NaCl (substituted with KCl when indicated), 10 Hepes, 5 KCl, 10 glucose, 1  $MgCl_2$ , 2  $CaCl_2$ . Intracellular: 140  $K^+$ -glutamate, 10 Hepes, 1 EGTA, 0.229  $CaCl_2$ , 1  $MgCl_2$ .

Again, the seals proved very stable and seal loss during suction pulses was a rare event (<10%).

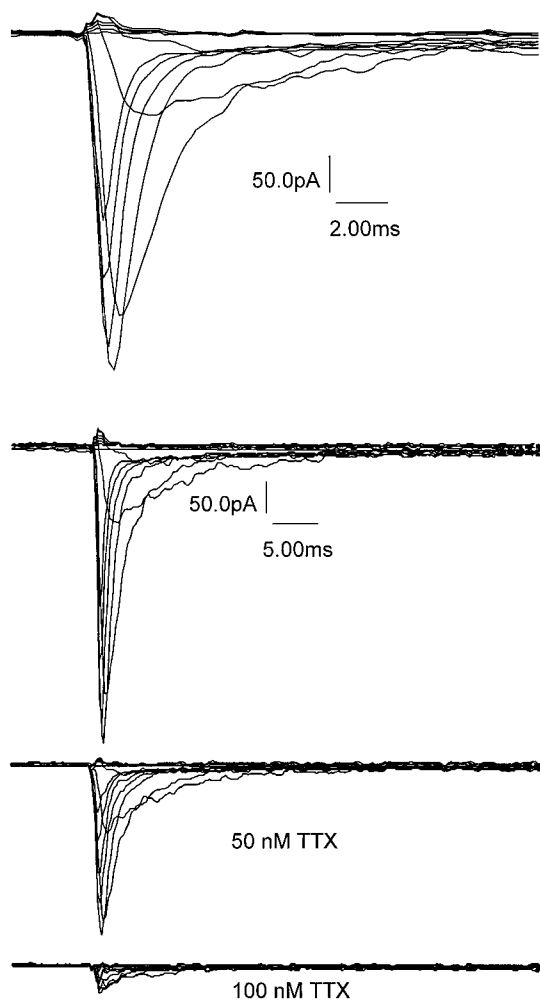
### LONG-TERM STABILITY OF WHOLE-CELL ACCESS

Long-term stability was a striking feature of whole-cell preparations corresponding to the high seal stability observed. More than 80% of the preparations had to be discarded after completion of the experiment (>15 min) with the seal still intact. Another striking difference when comparing the new method with conventional patch clamp was the low and stable access resistance, resulting in negligible voltage error and high voltage clamp quality. Using pipette tips with  $0.9 \pm 0.1$  MOhm tip resistance the series resistance estimated from capacity compensation settings was only  $2.7 \pm 0.5$  MOhm ( $n = 355$ , CHO cells) and remained completely stable during the typical 15 min experiment. This is in stark contrast to conventional patch clamp experiments, where series resistance after break-in tends to increase from  $\approx 5$  to  $\approx 20$  MOhm within 15 min depending on cell type and intracellular saline recipe (Penner 1995). This difference between our method and conventional patch clamp is probably due to the different geometry of the glass where the seal is formed. With conventional patch clamp, disrupted membrane fragments float inside the narrow pipette tip. When cells are sealed inside, the pipette taper has a larger diameter leaving more space for remnants of the membrane. Therefore, the

whole-cell preparation described here is ideally suited to compare current readings before and after addition of a drug during a screening run. Typical concerns about voltage errors and other artifacts developing over time with conventional whole cell experiments are virtually excluded. Furthermore, in an automated device, no repeated suction is required during the measurement of ionic currents, excluding seal loss and stretch-induced artifacts late in the experiment.

### PERFORATED PATCHES

In some instances chemical access to the cytosolic compartment is preferred in order to avoid washout of second messengers. This is typically achieved with ionophores such as nystatin or amphotericin and provides access for small ions only. The disadvantage over classical whole-cell preparations is the lack of



**FIG. 4.** Brain-type  $Na^+$  channels expressed in a CHO cell, sealed and opened by pressure pulse inside a pipette. The two upper panels show controls at different time scales. The lower panels show channel block with tetrodotoxin. Cells were held at  $-80$  mV and voltage steps incremented at 10 mV intervals up to 30 mV.

chemical control over the intracellular compartment, e.g., the application of  $K^+$  channel blocking ions for measuring  $Ca^{2+}$  channels is not feasible with perforated patches. We tested the ability to create perforated patches with different cell types sealed inside glass pipettes. When amphotericin was added to the tubing connected to the pipette tip, perforated patch access was achieved within 200 ms. Access resistance was in the order of 5–12 M $\Omega$  and seal stability was unaltered when compared to whole-cell preparations. An example for a recording obtained with perforated patches is shown in Figure 3.

### CLAMP QUALITY

In order to demonstrate the high quality of voltage clamp, we studied fast voltage dependent  $Na^+$  channels expressed in CHO cells. Figure 4 shows typical current traces and block of current using tetrodotoxin.

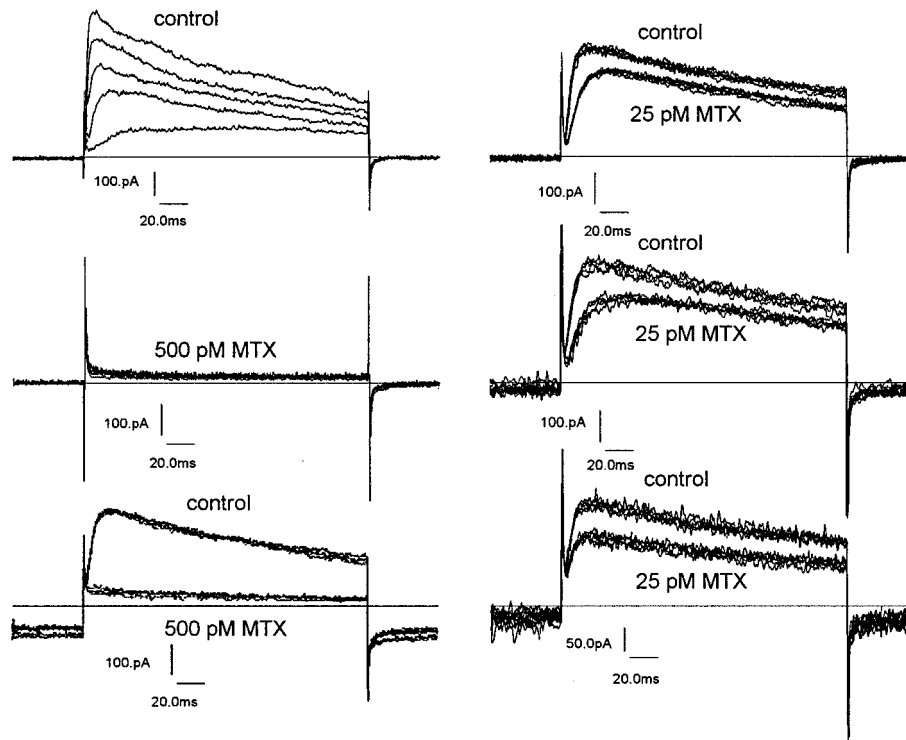
### FLUIDICS

A major advantage of the patch clamp configuration described here is the possibility to add compounds to the extracellular membrane surface using automated liquid handlers. The

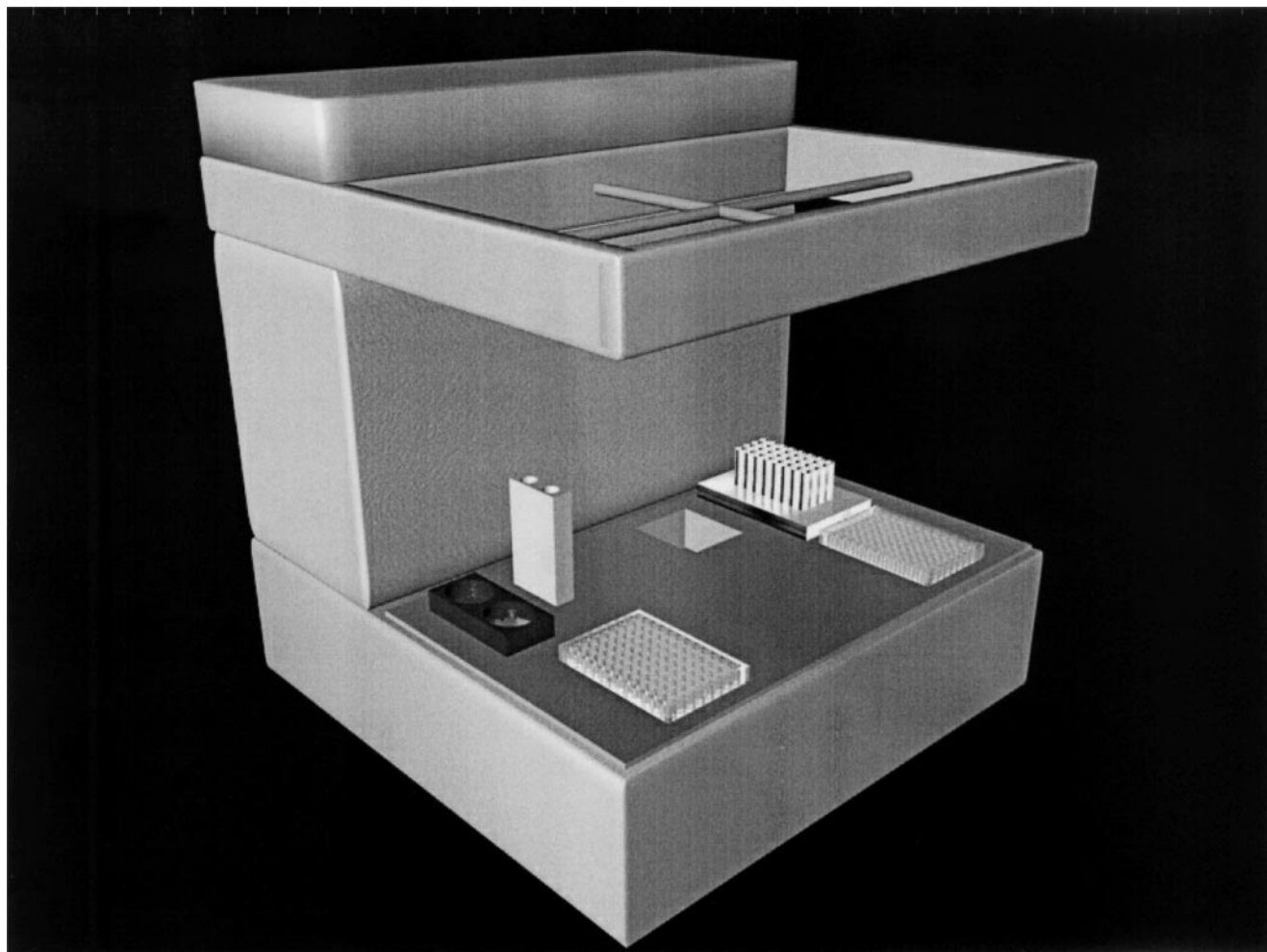
extracellular volume is small (15–20  $\mu$ l) and compound solution can be simply added and flushed into the wide opening of vertically mounted pipette tips from above. Figure 5 shows an example of an ion channel blocker reproducibly inhibiting whole cell currents. The high-affinity blocker of the  $K^+$  channel Kv1.3, margatoxin, was chosen to demonstrate the extreme sensitivity that only automated patch clamp can provide in the drug screening process, as well as the reproducibility of the results.

### ROBOTIC TIP ARRAYS

The method described here enabled us to design and develop a fully automated patch clamp robot that includes pipette and liquid handling, pressure manipulation, and amplifier electronics in a versatile desktop device. The core of the machine holds a number of receptacles for glass micropipettes in an array. Glass pipettes are embedded in a molded plastic jacket that facilitates pipette and liquid handling. The embedded pipettes are placed by the robot and filled with cell suspension. Seals and whole-cell preps are automatically obtained and the pipettes replaced until every single position in the array contains a functional whole-cell preparation. Control current is then taken, followed



**FIG. 5.** Kv1.3 channels in Jurkat T lymphocytes are blocked by picomolar concentrations of margatoxin. Examples from 5 different cells held at  $-60$  mV: (a) IV-relationship (lowest trace  $-20$  mV, 10 mV increment); (b) complete block in the same cell; (c) complete block in another cell (0 mV jumps) with traces overlaid that were taken 2 min apart in order to show long term stability; (d), (e), (f) partial block by 25 mM margatoxin in three different cells showing reproducibility and sensitivity.



**FIG. 6.** The “biochemist’s patch clamp” containing an array of pipettes and electrode positions. A vertical arrangement of pipettes allows addition of compound from the top. Pipettes are placed, sealed, measured and replaced independently. The process is fully automated.

by addition of compounds and, if desired, washout. The whole process is software-controlled and fully automatic. The operator needs only to replace trays containing embedded pipettes, compound plates, and refill cell suspension at given intervals.

The asynchronous measurements are performed at maximal throughput for each electrode position. The positions are completely independent, electrically separated, and shielded. No electrical cross talk between adjacent positions is observed. The machine can perform 300–1000 independent whole-cell screens per day.

#### “A BIOCHEMIST’S PATCH CLAMP”

Sealing cells inside narrow, tapered glass pipettes allowed the design of a robot that performs fully automated patch clamp experiments with high quality, high throughput, and low cost (Figure 6). No experience with micromanipulators is required. No biophysics/electronics wizard is needed. This type of machine has recently been dubbed a “biochemist’s patch clamp” (Sigworth and Klemic 2002), and at present various companies

introduce machines based on different technologies. More cell biologists and pharmaceutical researchers will turn their attention to ion channels since this protein class has been shown to be linked to various diseases and pharmaceutical safety problems. The methods allowing automated patch clamp measurements will revolutionize the drug screening process and profoundly accelerate scientific and commercial advances in the field of membrane transport.

#### REFERENCES

- Fertig, N., Blick, R. H., and Behrends, J. C. 2002. *Biophys. J.* 82:3056–3062.  
 Klemic, K. G., Klemic, J. F., Reed, M. A., and Sigworth, F. J. 2002. *Biosens. Bioelectron.* 17:597–604.  
 Numann, R., and Negulescu, P. A. 2001. *Trends Cardiovasc. Med.* 11:54–59.  
 Penner, R. 1995. A practical guide to patch clamping. In *Single channel recording*, ed. B. Sakmann and E. Neher. New York and London: Plenum Press.  
 Sakmann, B. and Neher, E. 1995. Geometric parameters of pipettes and membrane patches. In *Single channel recording*, ed. B. Sakmann and E. Neher. New York and London: Plenum Press.  
 Sigworth, F. J., and Klemic, K. G. 2002. *Biophys. J.* 82:2831–2832.