What is patch clamping and what is it used for?

Patch clamping is used to measure ion currents across biological membranes. In our laboratory we measure currents through K$^+$ channels and anion channels in guard cells and mesophyll cells isolated from *Vicia faba* and *Arabidopsis thaliana*. We are interested in how different compounds like Abscisic Acid, Ozone and G-protein regulators affect ion channels.

How does it work?

To gain access to the plasma membrane we isolate protoplasts with the help of enzymes that digest away the cell wall (except laser patch clamping, see below). These naked cells, called protoplasts, are kept in a solution (bath solution) with appropriate osmolarity and various ions depending on which ion channels we are interested in and experimental design. A glass pipette is filled with a pipette solution and an Ag/AgCl wire connected to an electrical device called the patch clamp (amplifier). The patch clamp is connected to a computer so we can control experimental parameters and analyze the acquired patch clamp data. The patch pipette is moved to the surface of the protoplast and mild suction is applied to obtain a gigaohm seal between the pipette and the plasma membrane. From there various approaches can be used (see patch clamp configurations below).

Ion currents are converted into electrical currents and vice versa at the Ag/AgCl wire, so that ion currents going across the membranes can be read as electrical currents by the patch clamp. We use a technique called voltage clamping where the membrane potential of the cell is held constant (clamped) while we measure ion currents across the membrane.

Bath dish with ground and pipette electrode mounted on the microscope:
*Vicia faba* guard cell protoplast being approached by the patch pipette:

**Patch clamp configurations**

**Whole cell patch clamping** is used when we want to measure the average current across the entire surface area of one cell. The pipette is moved to the surface of the membrane and suction is applied to achieve a gigaohm seal between the glass pipette and the membrane. The small piece of membrane surrounded by the pipette tip is thereafter ruptured and equilibrium is obtained between the pipette solution and the cell content. The ion concentrations on both sides of the membrane are known and used to calculate Nernst potential for each ion, which helps us elucidate which ions contribute to the measured currents.

Solutions are chosen to enhance the current through the ion channels of interest, e.g. $K^+$ being the main ion when studying $K^+$ channels. Typically a series of membrane potentials, called a voltage family, is applied while ion currents are measured. Examples of recordings are shown below.

$K^+$ currents measured from *Arabidopsis thaliana* guard cell:

Anion currents measured from *Vicia faba* guard cell:
Single channel patch clamping is a technique with very high resolution. Conformational changes in one single protein can be detected; the opening or closing of one ion channel.

There are 3 configurations of the single channel patch clamp technique: cell attached, inside-out patch and outside-out patch. With cell-attached configuration recording of currents is done after a gigaohm seal is obtained between the pipette and the membrane. The cell content is intact, but it is tricky to figure out which ions contribute to the current since the ion concentrations inside the cell are unknown. Inside-out patches are obtained by first getting a cell-attached seal and then pulling the glass pipette away from the cell so that a small piece of membrane is attached to the pipette with the cytosolic side facing the bath solution. This configuration is preferred when you are studying how cytosolic events affect an ion channel. Out-side out patches are obtained by first achieving the whole cell configuration and then pulling the pipette away from the cell so that the external side of the membrane is facing the bath solution. Out-side out patches are appropriate when studying effects occurring on the external side of the membrane.

In all single channel configurations different membrane potentials are applied to the patch and ion currents through one or a few ion channels can be observed as they open and close in a step-like manner (see example below).

Recording from an out-side out patch with one single $K^+$ channel:

--- open channel

--- closed channel

Good article if you want an easy explanation of how to understand patch clamp data:

Laser patch clamping

This is a specialized form of patch clamping. Instead of applying enzymes to digest away the cell wall, a laser is used to make a small hole in the cell wall while the cells are plasmolysed. After the hole is made, solutions are changed so that the cells are deplasmolysed and a small membrane bleb comes out of the hole in the cell wall. The patch pipette is then moved to the membrane bleb to obtain a gigahm seal. Cell-attached single channel recordings or whole cell patch clamp recordings can be performed with laser patch clamping.

Laser patch clamp papers from our laboratory:

