K^+-channel traffic to the plasma membrane

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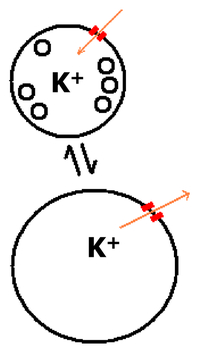
1. Correlating Membrane and Ion Channel Traffic

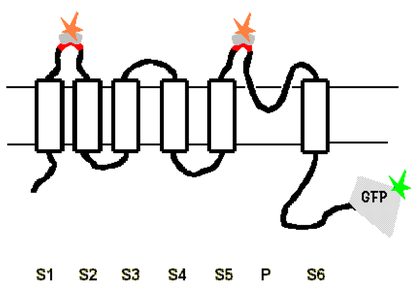
Shrinking and swelling of guard cells, which leads to the opening and closing of the stomatal pore is driven by ion currents across the plasma membrane and involves addition and retrieval of membrane.

So ion channels may undergo considerable traffic between compartments in this model system.

We labelled a guard cell channel to track it's whereabouts in the cell.



2. Labelling an Ion Channel



Shown here is a sketch of the *Arabidopsis* potassium inward rectifier KAT1 double labelled with hemagglutinin (HA) epitopes facing the apoplast and with GFP at the C-terminal end.

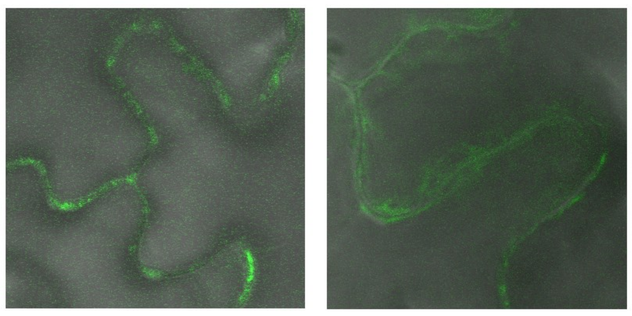
This labelling scheme allows tracking the protein at different stages within it's cycle.

The alterations do not affect the electrophysiological characteristics of the KAT1 channel.

3. Expression Patterns

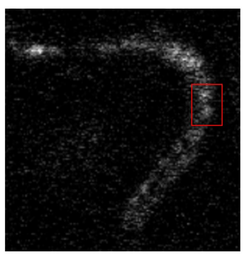
Using Agrobacterium, we expressed the mutated KAT1 channel transiently in tobacco epidermis cells.

In low expressing cells, the GFP signal is only visible along the outline of the cell.
If the expression level is higher we also find cytoplasmic strands carrying the GFP label.



4. Clusters of Channels

Often the KAT1 channels appear in the plasma membrane or in the vicinity of it in small clusters.

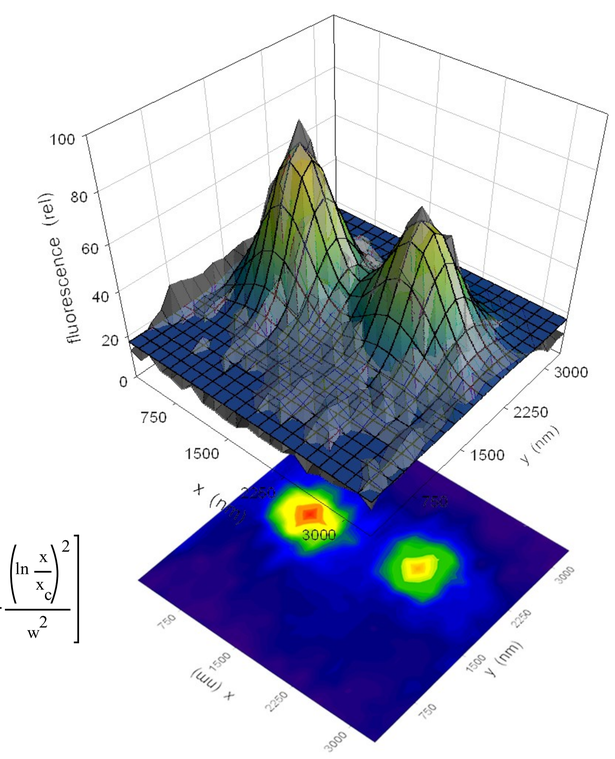


To measure the size of the fluorescent clusters, we took clearly distinguishable clusters from each image fitting them with a spherical Gaussian Distribution in the form:

$$f(x,y) := -I_o + A \cdot e^{-\frac{1}{2}\cdot\left[\left(\frac{x-x_o}{w_x}\right)^2+\left(\frac{y-y_o}{w_y}\right)^2\right]}$$

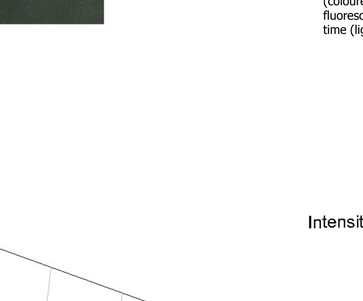
With I_o the background fluorescence and w being the half maximal peak height in x and y direction.

Calculating the mean size using: $f(y) := -y_o + A \cdot e^{\left[-\frac{\left(\ln\frac{x}{x_c}\right)^2}{w^2}\right]}$ yields a mean cluster size of 450 nm; in the range of values obtained by Meckel et al. in *vicia* guard cells [2] using this type of analysis.

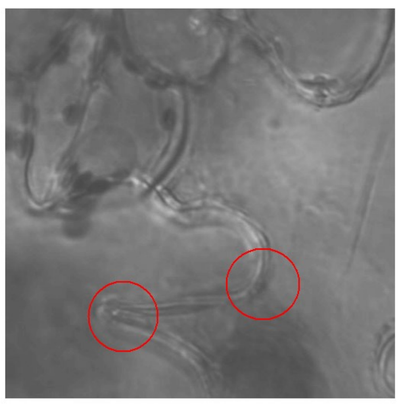


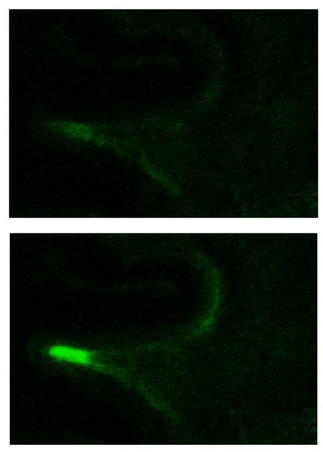
5. Light Activatable GFP

Green Fluorescent Protein (GFP) is of great use in localisation of proteins. Even movement can be traced when clusters of protein move against a dark background. Movement of proteins within a relatively homogeneous population though is hardly visible using GFP.



We used a light activatable form of GFP (GFP-la) that does not normally show fluorescence. Under irradiation with UV light the GFP undergoes a photoconversion into an 'active' state and can be used like a normal GFP variant [1].



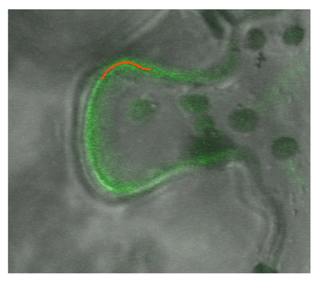


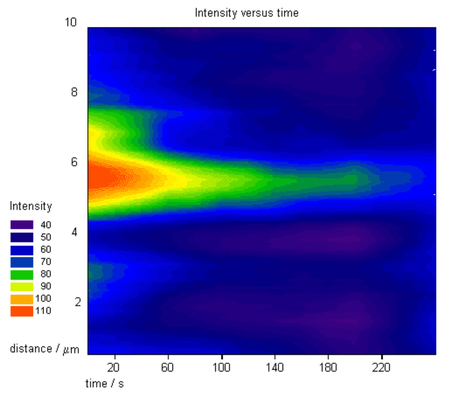
6. Putting numbers to movement

Confocal Micrographs allow pinpointing the location of a labelled protein within a cell with some certainty. Compartments though, which are in close proximity, can well yield misleading locations. Analysis of protein mobility may allow distinguishing between compartments.

In every consecutive picture taken of a cluster of KAT1 channels, the spot seems to be less pronounced and sometimes widening along the membrane.

Are we looking at a cluster of channels spreading by diffusion?

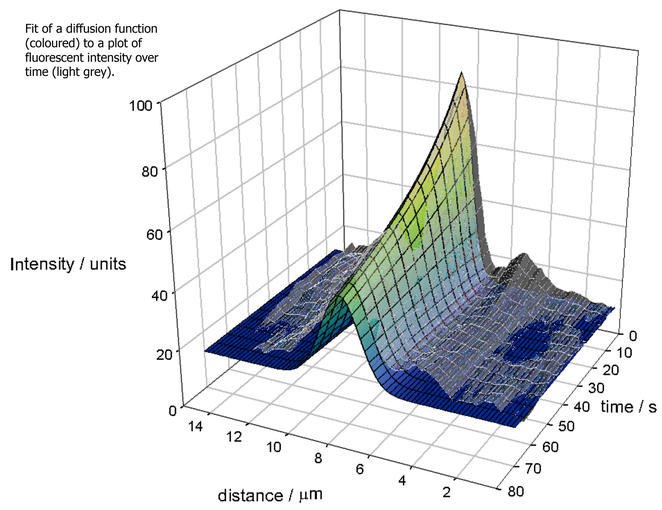




Diffusion of a point source can be described using Green's Function:

$$f(x,t) := \frac{C_0}{\sqrt{4\cdot\pi\cdot D\cdot t}}\cdot e^{\frac{-x^2}{4\cdot D\cdot t}} + \Delta t$$

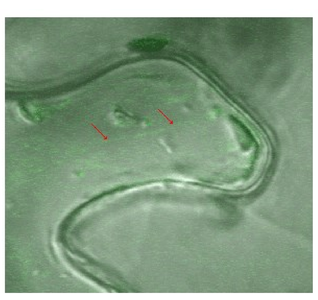
with x being the translocation distance, D the diffusion coefficient, C_0 the initial concentration of GFP and Δt an offset accounting for the initial size of the spot.

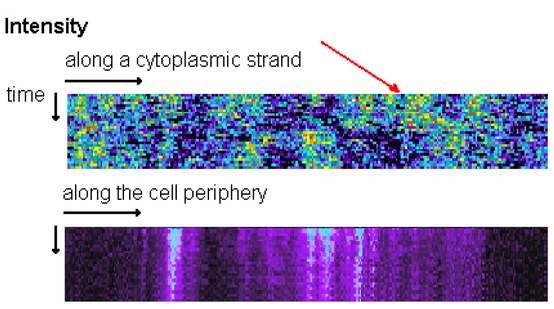


Fits to clusters over time yielded diffusion coefficients
$D < 3\ 10^{-15}\ m^2s^{-1}$.

The offset Δt fits at 30 to 60s, the initial concentrations were found to be in the range of $2\ 10^{-8}$.

7. Movement within the Cytoplasm





While the K^+ channels within the plasma membrane do move comparatively slow (6.), there is fast traffic within the cytoplasm.

A plot showing the fluorescent intensity over time along the cell periphery and along a cytoplasmic strand (red arrows), shows a nearly static picture at the cell surface but a very dynamic pattern within the cell. In few cases a very bright spot can be traced through some few images (red arrow) but in general the movement is to fast to be recorded by high resolution confocal microscopy over larger areas.
The KAT1 channels move along cytoplasmic strands at speed in excess of 0.5 µm s^{-1}.

References:

[1] George H. Patterson & Jennifer Lippincott-Schwartz, A Photoactivatable GFP for Selective Photolabelling of Proteins and Cells, 2oo2, SCIENCE 297, 1873-1877
[2] Tobias Meckel, Annette C. Hurst, Gerhard Thiel & Ulrike Homann, Endocytosis against high turgor: intact guard cells of *Vicia faba* constitutively endocytose fluorescently labelled plasma membrane and GFP-tagged K^+-channel KAT1, 2oo4, The Plant Journal, 39, 182-193

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