

Activation of CFTR channels in absence of internal fluoride using a highly parallel automated patch clamp system

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Abstract

Cystic fibrosis is caused by malfunction of the chloride channel Cystic Fibrosis Transmembrane Regulator (CFTR). CFTR is expressed in the apical membrane of epithelial cells where it is involved in the regulation of fluid transport across the epithelium. A large number of mutations of the protein are known to cause CFTR to become dysfunctional and only very few pharmaceutical compounds have been developed to treat the disease by restoring the chloride conductance of the channel. CFTR is activated by cAMP dependent phosphorylation and is gated by ATP. Activation is typically achieved using forskolin that activates adenylate cyclase which leads to phosphorylation of the channel via protein kinase A (PKA). An alternative mode of activation is via application of intracellular F⁻. However, intracellular F⁻ has also been widely used on most automated patch clamp (APC) platforms due to it's stabilizing effects during the giga-seal formation. On the other hand, this also makes it difficult to control the degree of activation of the channel and represents a general limitation in the use of APC platforms in drug discovery for CFTR.

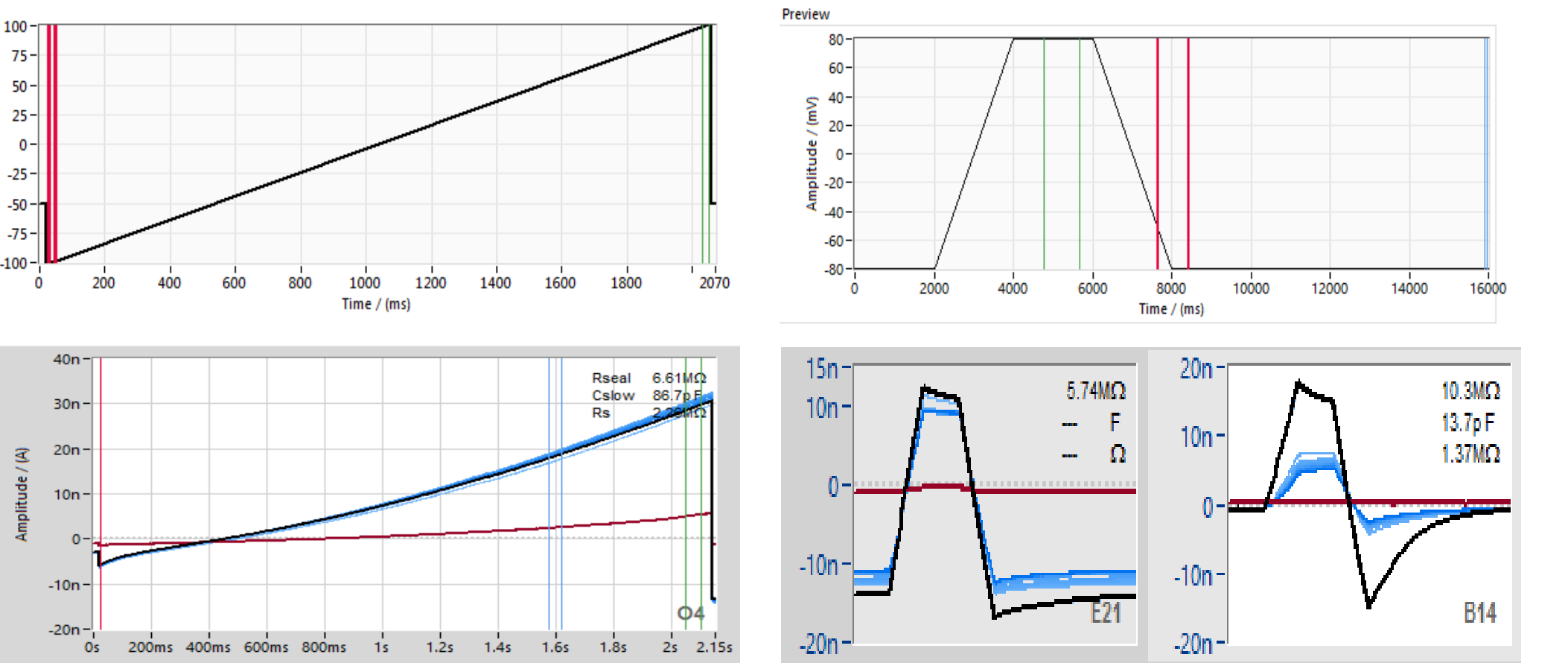
We now here present data from Nanion's SyncroPatch 384PE where we activate the channel with forskolin under F-free conditions at a high throughput. Further, CFTR could also be activated by first patching the cells in F-free conditions and then exposing the cell to intracellular F⁻ using the internal solution exchange function. Our results show that the activation of CFTR by either forskolin or intracellular F⁻ is sensitive to Glibenclamide in a dose – and voltage dependent manner. Taken together, these experiments firstly show a fluoride-free approach to study the pharmacology of CFTR at high throughput that might empowers new ways in the drug discovery on CFTR.

Methods

Cells
The experiments presented here were done using CHO cells expressing wild type CFTR kindly provided by Charles River. The cells were cultured according to the manufacturers recommendations and harvested according to Nanion's standard protocol using trypsin.

Voltage protocols
CFTR currents were monitored using a 2 second ramp protocol from -100 to 100 mV every 15 seconds. A second longer ramp protocol was also used later in the experiment series (see below) where the voltage was ramped down again to the holding potential of -80 mV. This protocol allowed to distinguish the proportion of unblocked current as opposed to leak in the full block period.

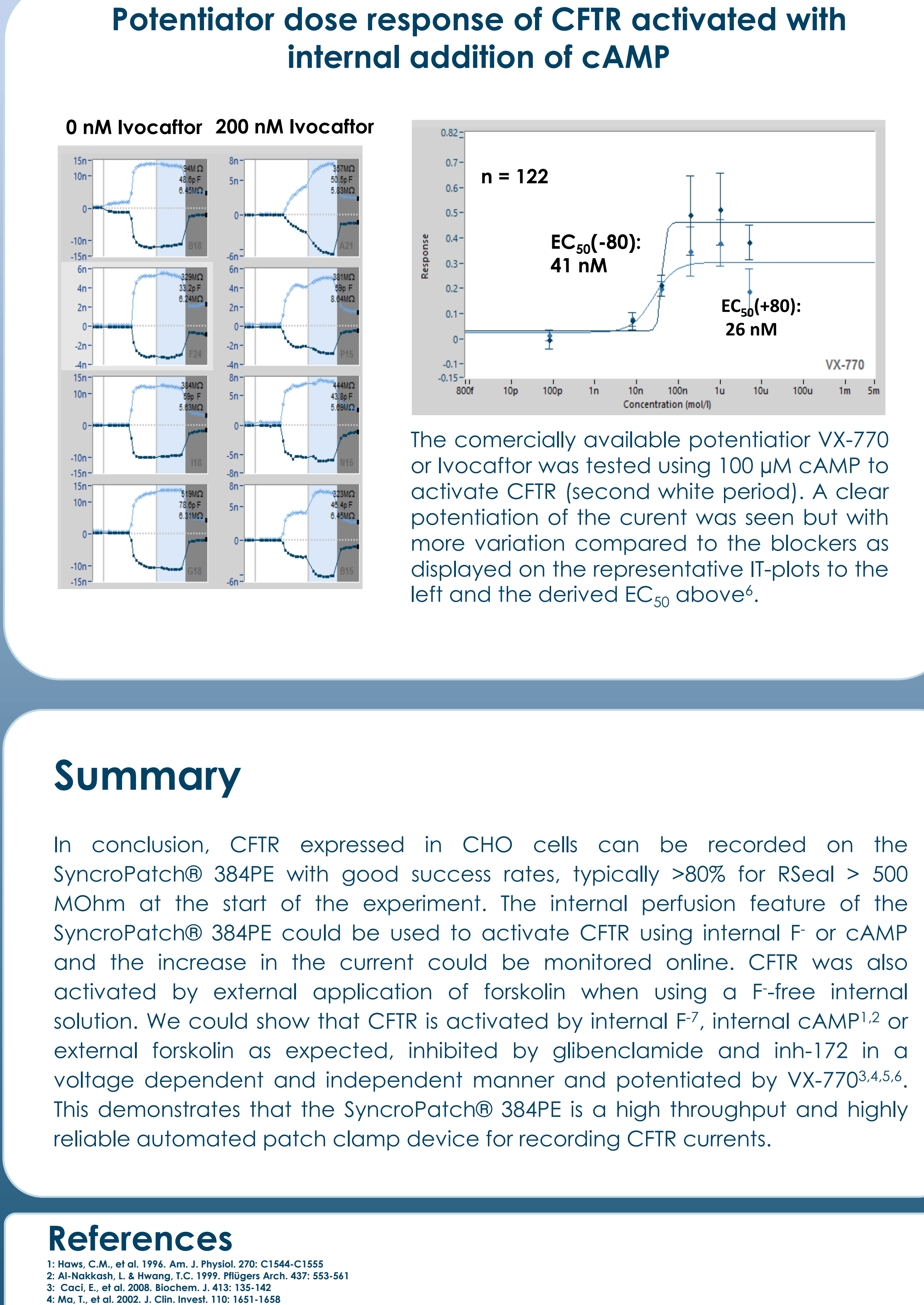
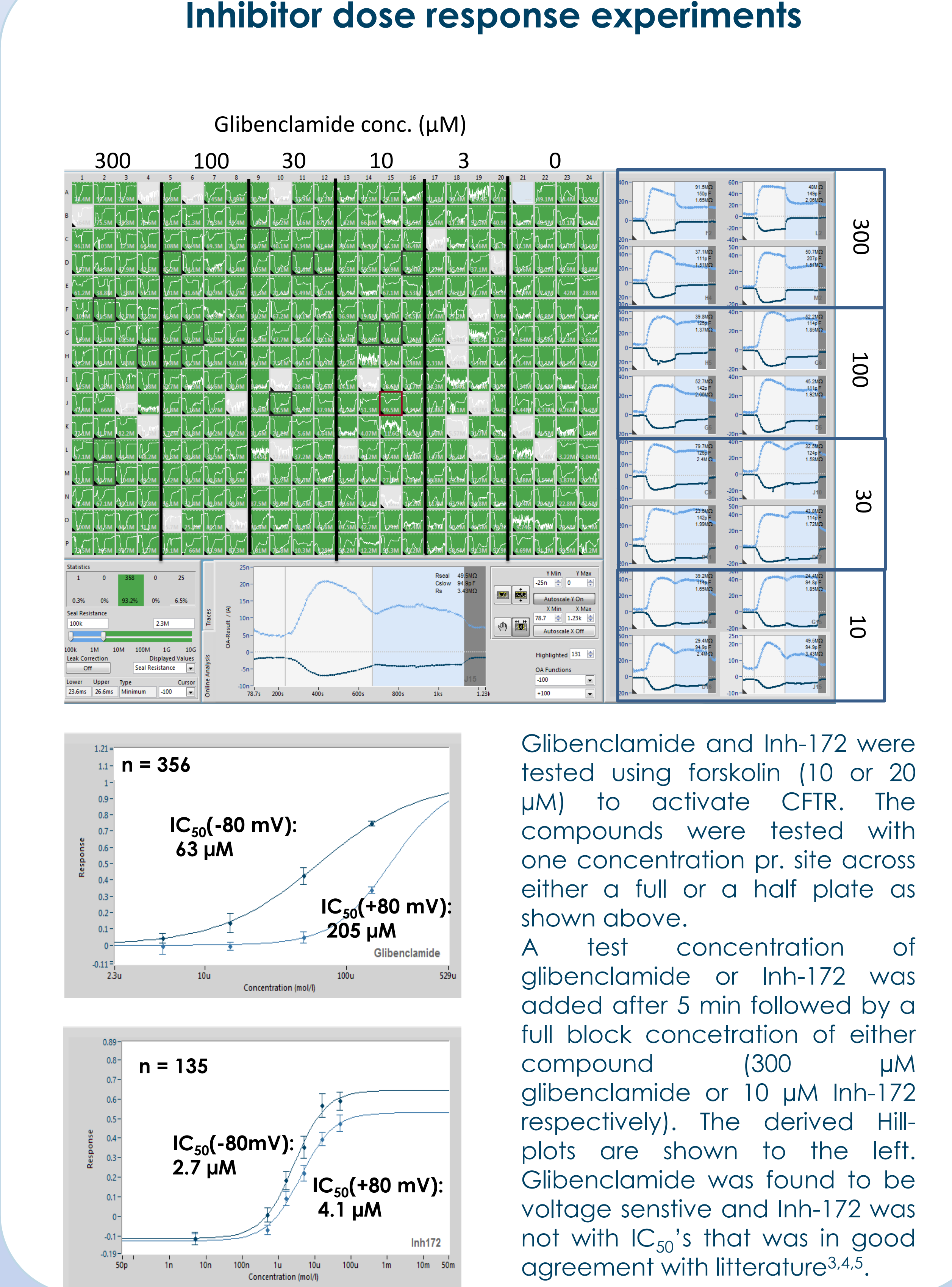
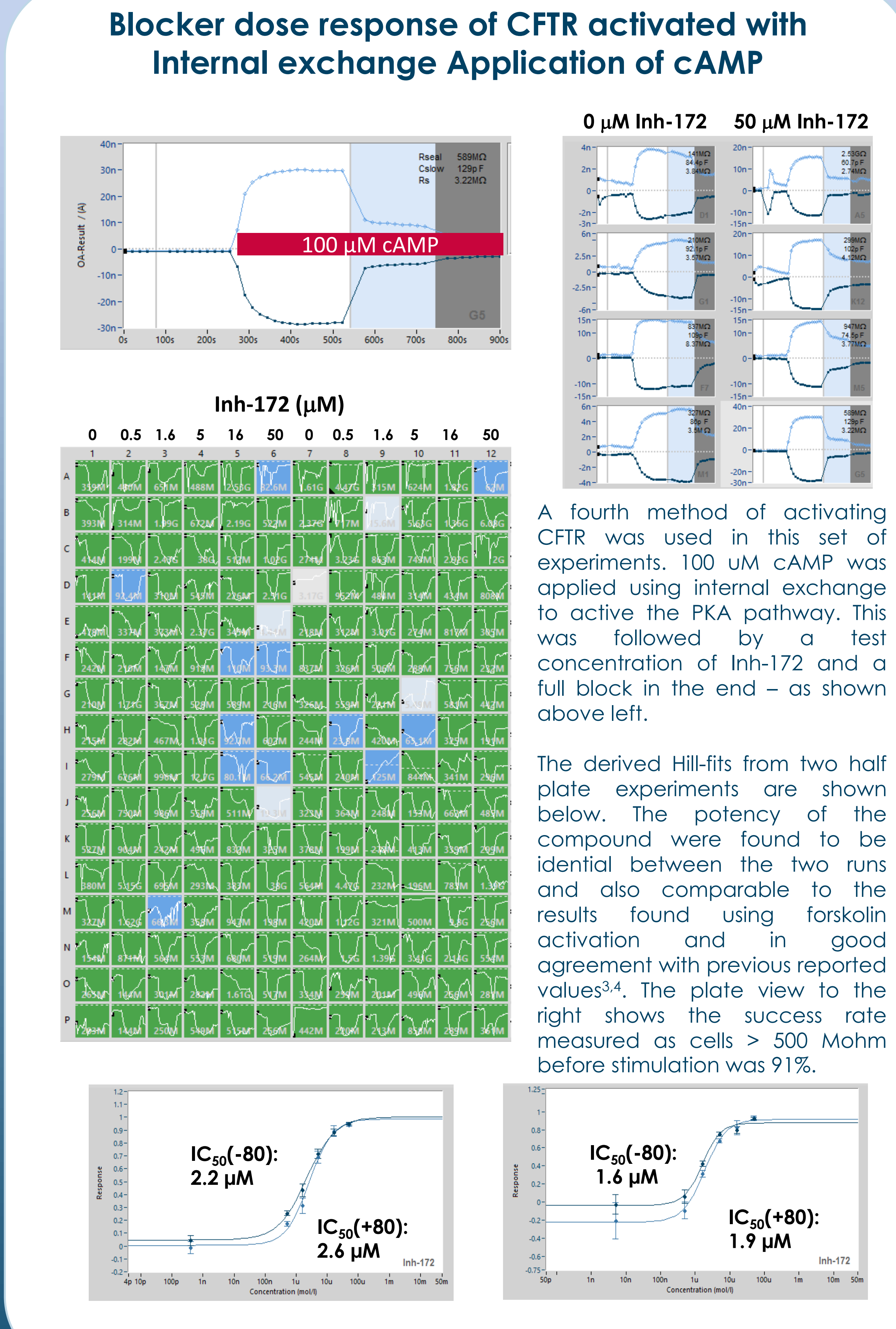
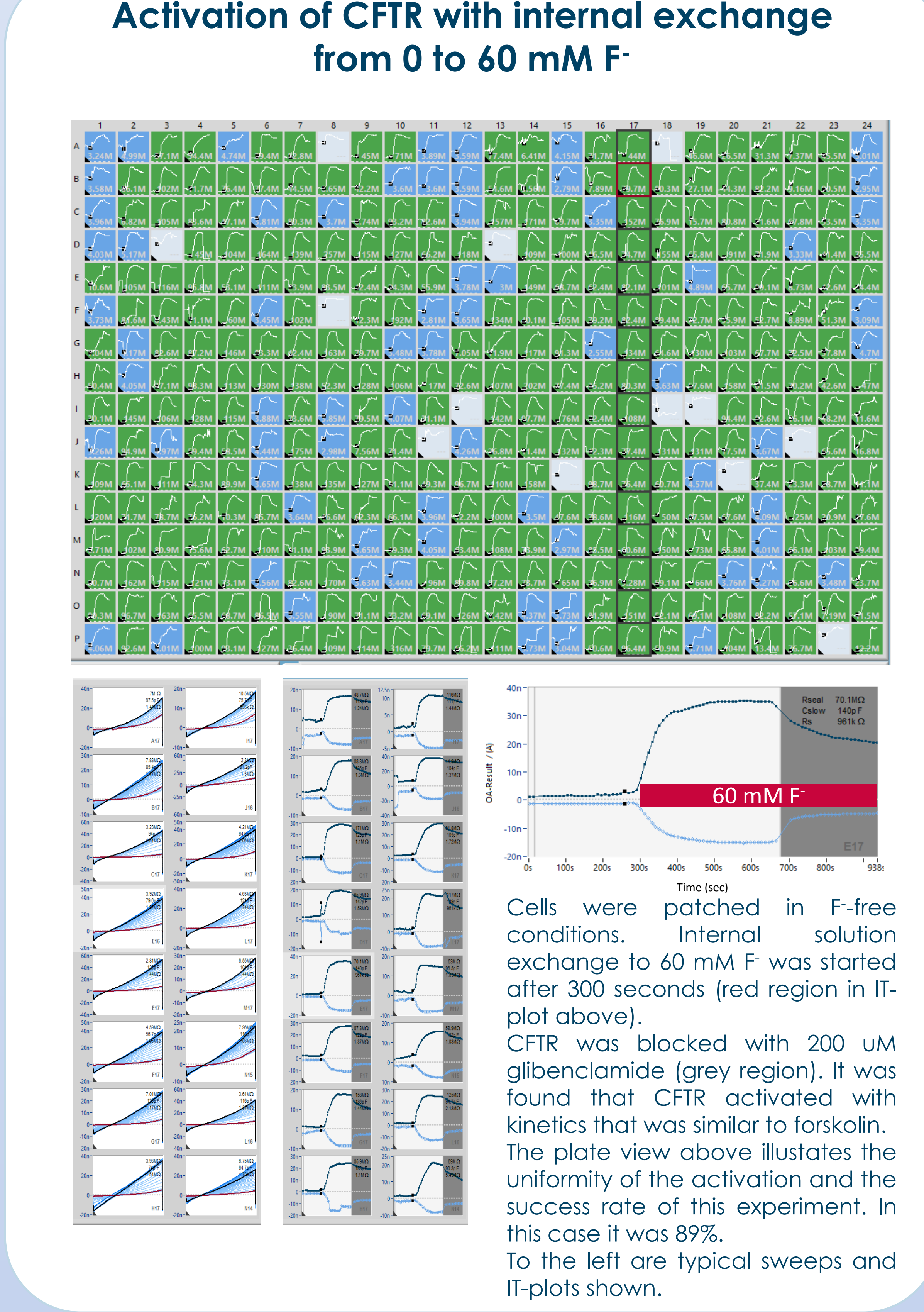
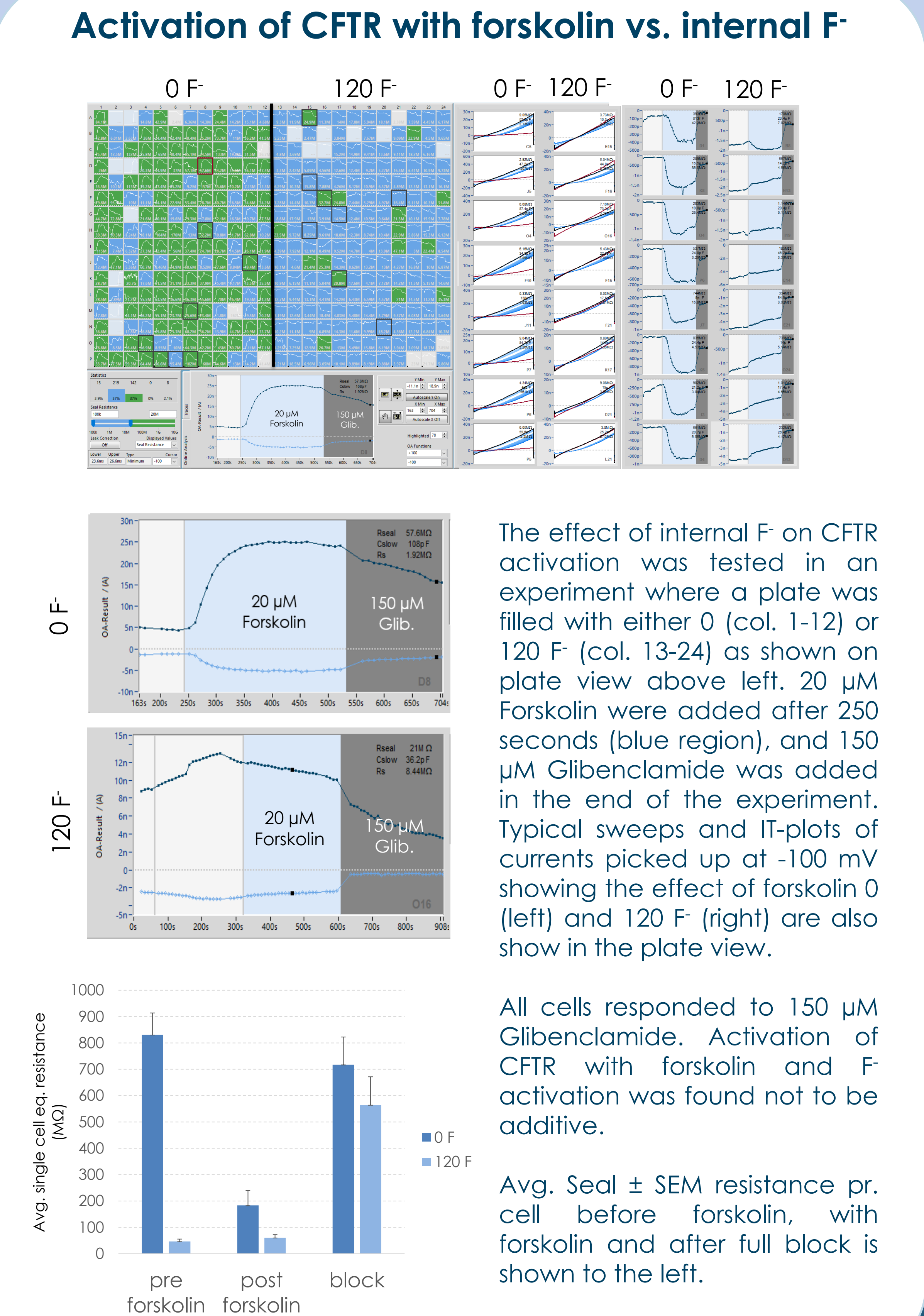
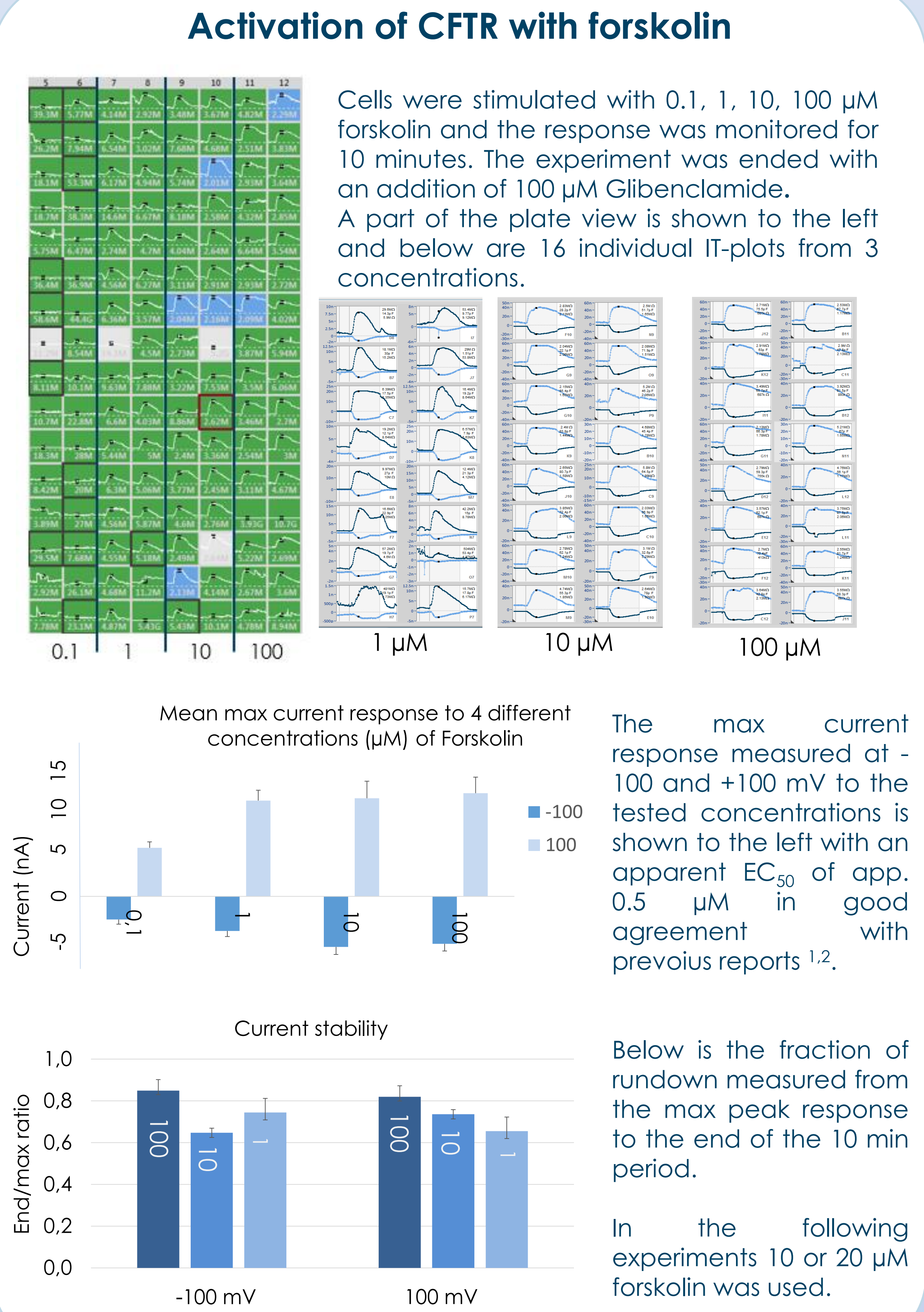
Electrophysiology
Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE using multi-hole chips. All experiments followed a typical sequence: filling and patching and whole cell formation followed by a baseline recordings for 60-120 seconds, an activation period with either internal exchange and/or external addition of forskolin, an addition of test compound for 3-5 minutes which was followed by full block of either glibenclamide or Inh-172. Total experiment lenght including filling and patching was app. 20 minutes.



Used voltage protocols and typical current responses. In red is preactivation, in dark activated current. On the right are two examples of a leaky (left) and a non-leaky (right) well. Note the difference in tail current revealing leak vs. residual current. CFTR was measured at ±100 or 80 mV respectively.

- ✓ 384 Amplifiers, 384 Pipetting Head
- ✓ High Giga-seal rate on HEK, CHO, LTK, RBL, iPS and RBC
- ✓ Fast solution exchange for ligand gated channels
- ✓ Partial Usage of the Chips Possible (32 wells per run)
- ✓ Temperature controlled cell hotel (4 - 70 °C)
- ✓ Customized Chips for different hole sizes, allowing optimal access and seal resistances for different cell lines
- ✓ Upgradable Current Clamp Option
- ✓ Upgradable Temperature Control Option
- ✓ Internal Solution Exchange

SyncroPatch® 384PE



Summary

In conclusion, CFTR expressed in CHO cells can be recorded on the SyncroPatch® 384PE with good success rates, typically >80% for RSeal > 500 MOhm at the start of the experiment. The internal perfusion feature of the SyncroPatch® 384PE could be used to activate CFTR using internal F⁻ or cAMP and the increase in the current could be monitored online. CFTR was also activated by external application of forskolin when using a F-free internal solution. We could show that CFTR is activated by internal F⁻, internal cAMP^{1,2} or external forskolin as expected, inhibited by glibenclamide and inh-172 in a voltage dependent and independent manner and potentiated by VX-770^{3,4,5,6}. This demonstrates that the SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording CFTR currents.

References

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