

High throughput real-time measurement of electrogenic membrane transport driven by the SLC transporters PepT1 and Oct2.

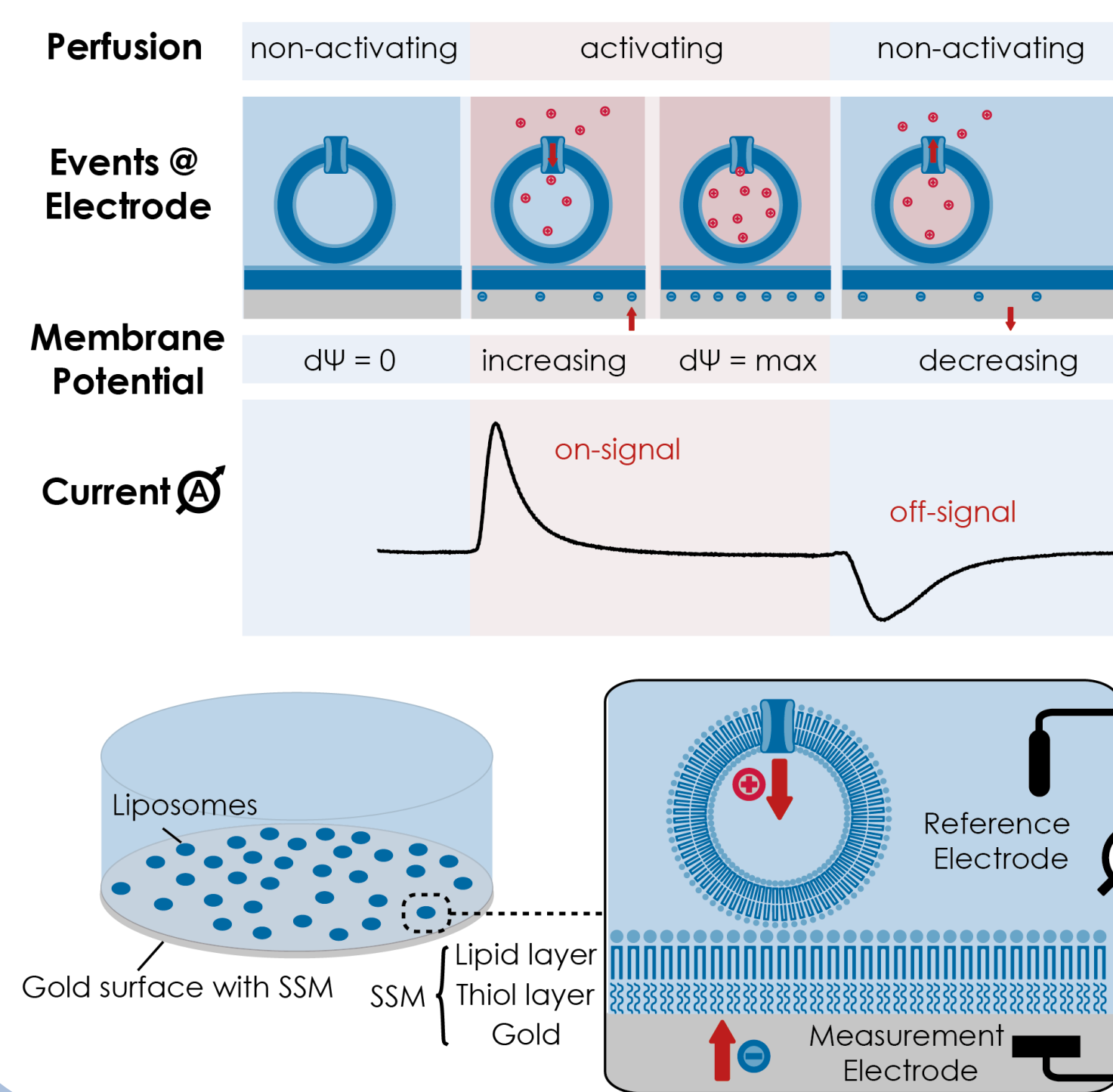
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Maria Barthmes¹, Andre Bazzone¹, Stephan Holzhauser¹, Niels Fertig¹, Michael George¹, Andrea Brüggemann¹

¹Nanon Technologies GmbH, Gabrielenstr. 9, 80636 Munich, Germany, contact: Info@nanion.de

An electrophysiological method suitable for transporters and pumps

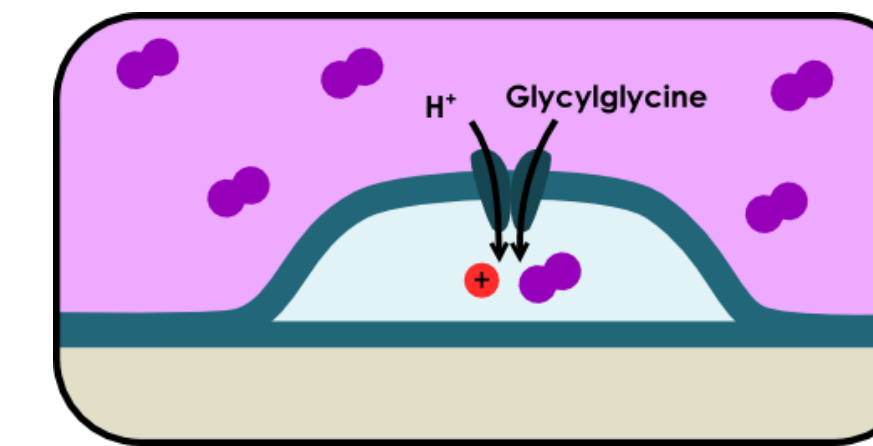
“Solid Supported Membrane (SSM) based electrophysiology” is an established method, developed at the Max-Planck-Institut, which allows to record low amplitude electrogenic events with a high amplification. The method allows real-time activity measurement of targets for which conventional electrophysiology is unsuitable, like many electrogenic membrane transporters and pumps, or targets localized in intracellular or bacterial membranes.



This is achieved by the use of a 1-3 mm gold electrode which is coated with an SSM and membrane vesicles or liposomes containing the target protein. The protein of interest is activated by a fast solution exchange. In electrogenic transporters this generates a membrane potential, which can be measured at the electrode. The peak current is proportional to the turnover rate.

PepT1/ SLC15A1

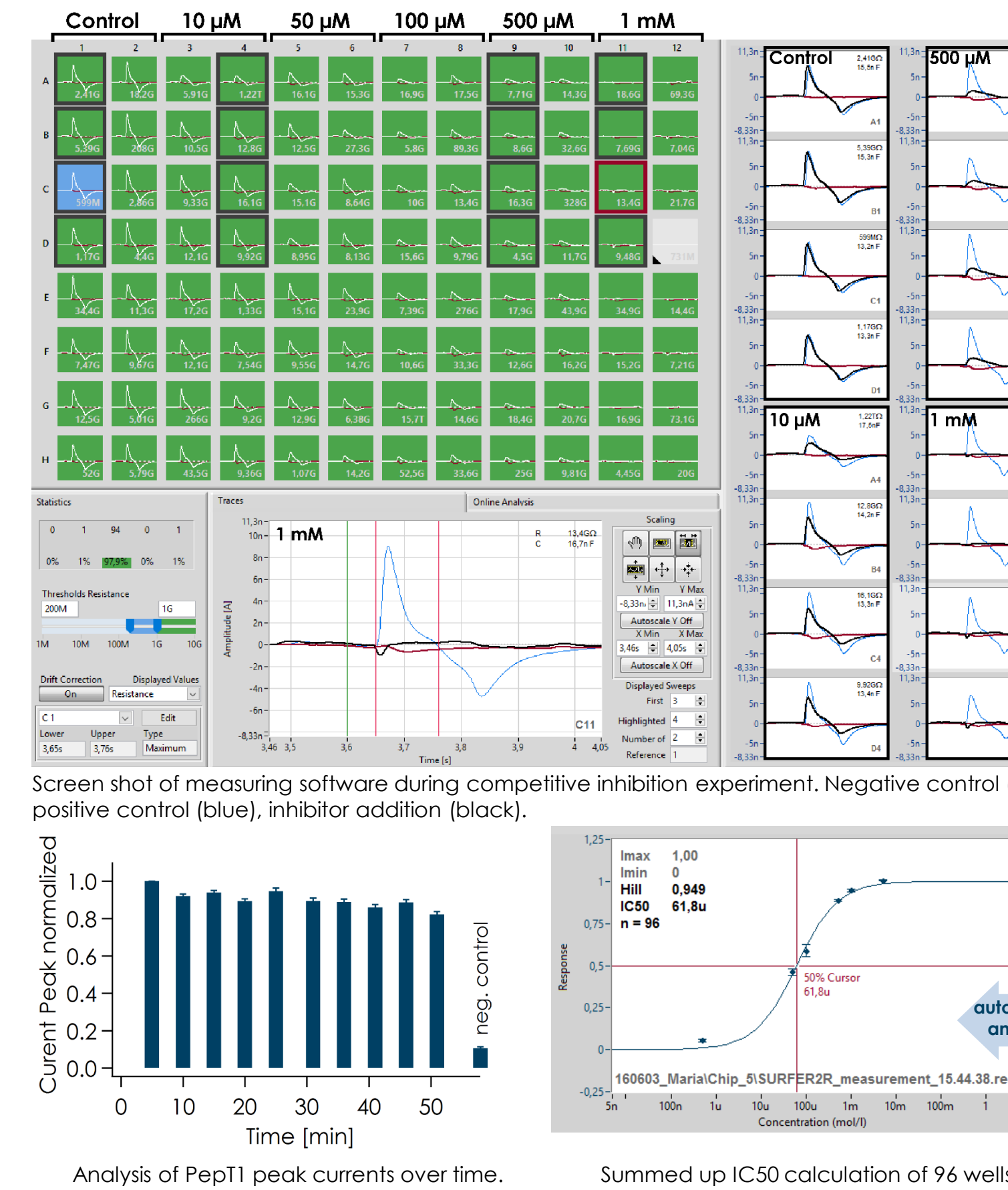
Function: Absorption of oligopeptides in the small intestines
Mechanism: Proton/Peptide symport into the cell
Clinical significance: Increasing the bioavailability of drugs



Competitive Inhibition.

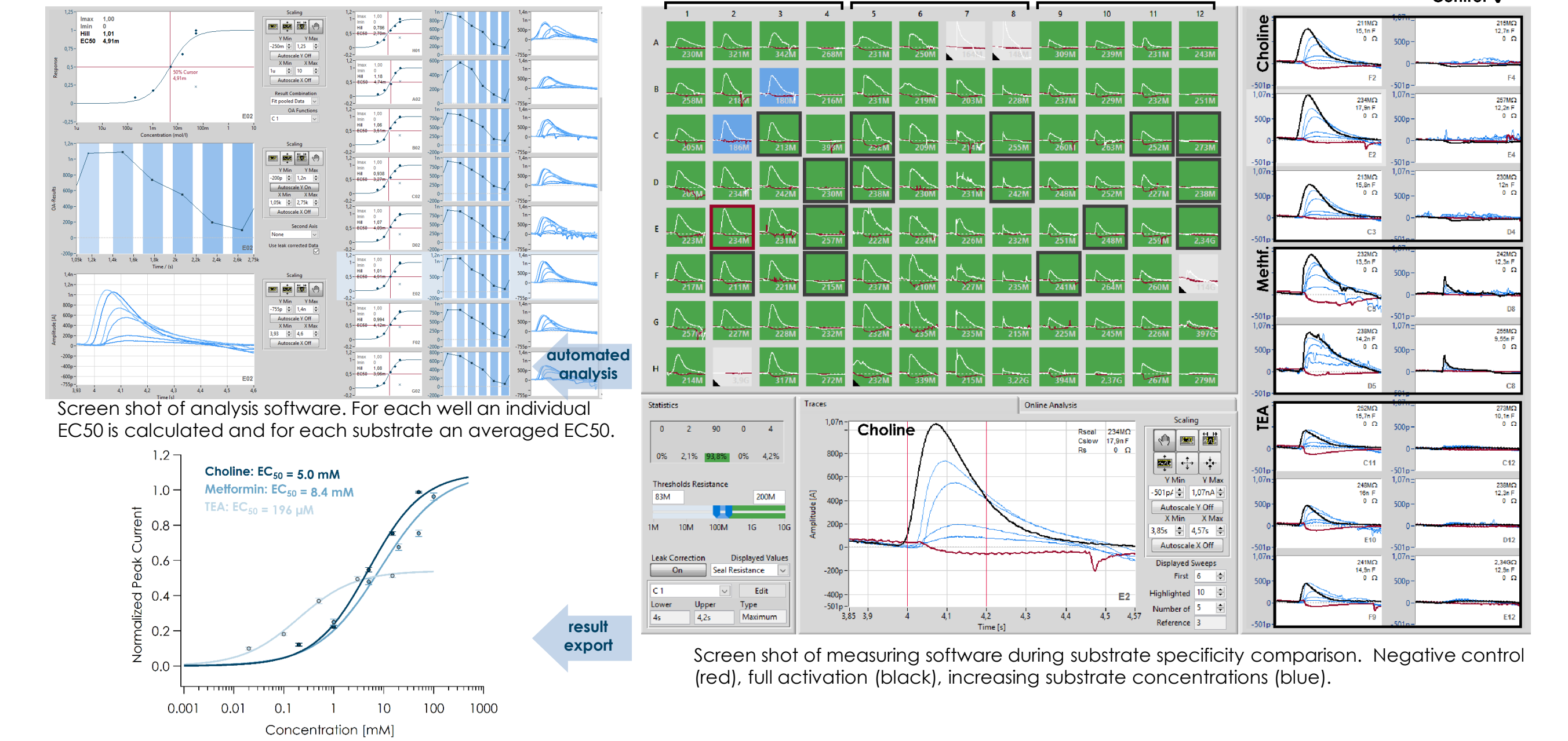
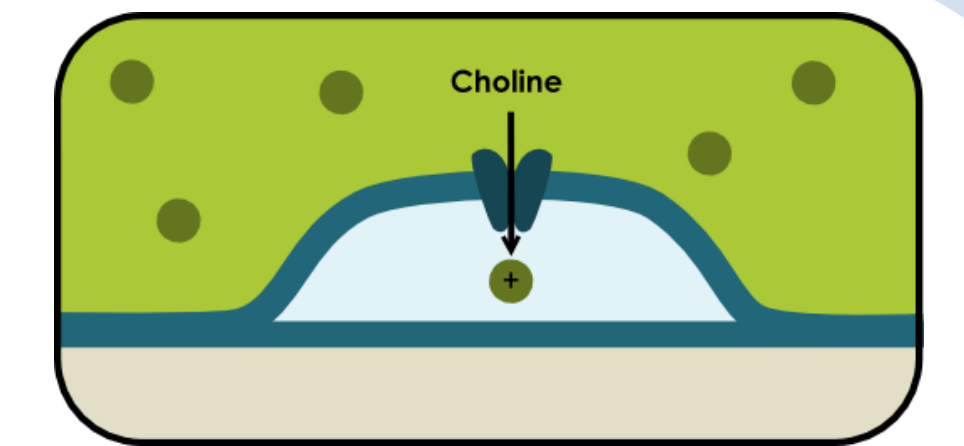
PepT1 was expressed in CHO cells and the membrane was purified. IC₅₀ determination: An inhibitor is applied in various concentrations. In advance, a negative (red) and a positive control (blue) were performed in each well. The full experiment takes 15 min.

Signal Stability. Repeated activation of PepT1 demonstrates stable signal amplitudes for more than an hour. This enables recordings of replicates and cumulative compound addition.



OCT2/ SLC22A1

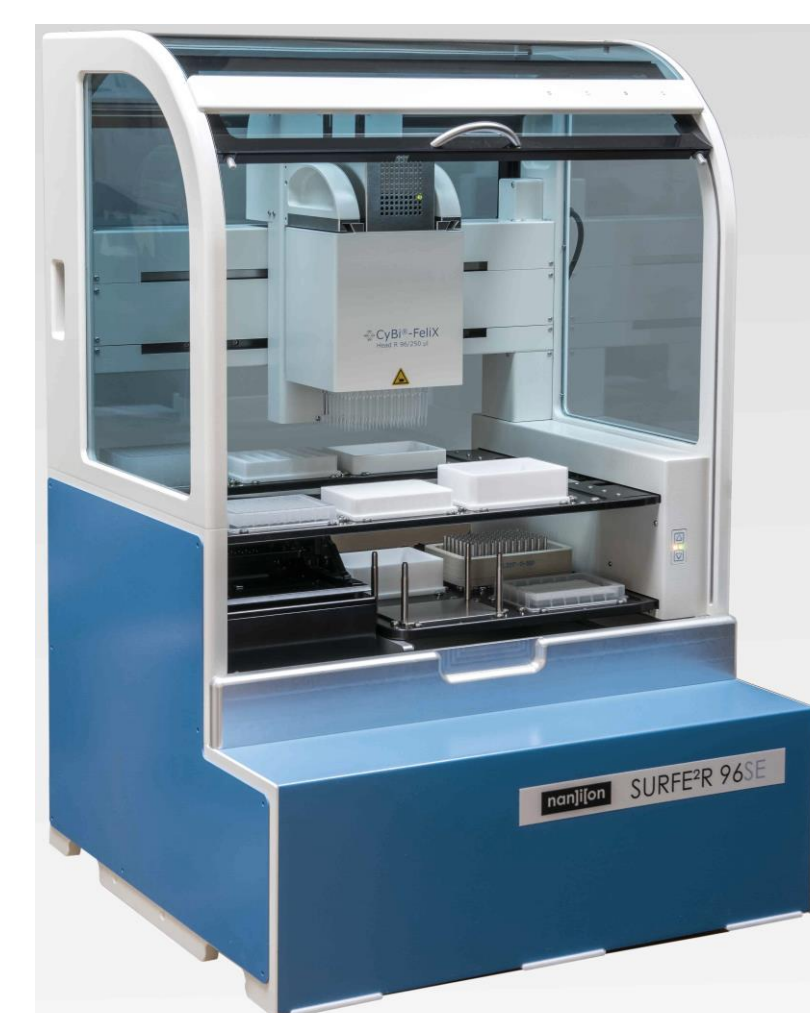
Function: Renal secretion of organic cations
Mechanism: Polyspecific cation transport
Clinical significance: Renal clearance, drug interactions



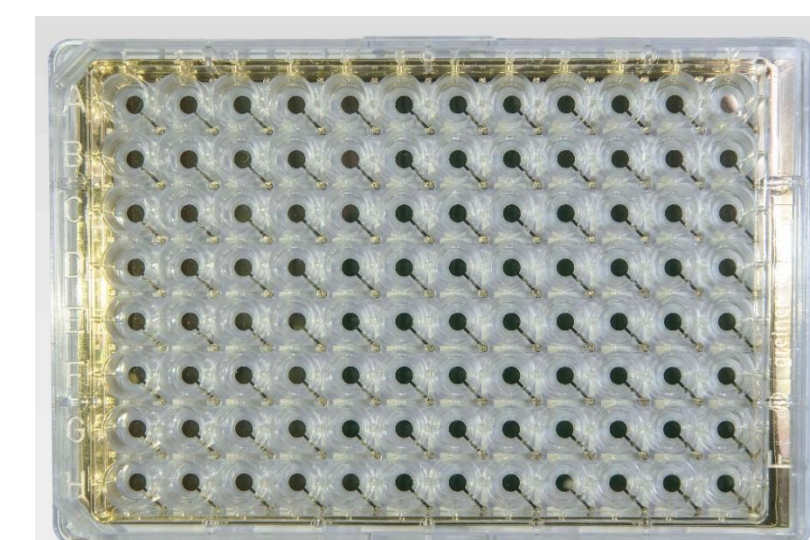
Substrate specificity.

OCT2 was expressed in CHO cells and the membrane was purified. Three different substrates are provided in increasing concentration cumulatively in each well. The substrates show different kinetics and affinities. Parental membrane was used as a negative control.

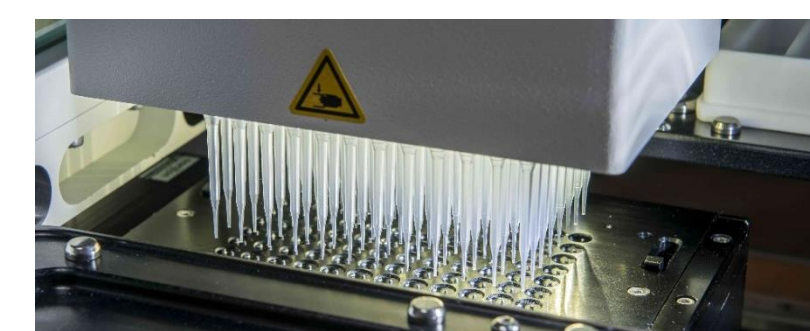
Scale-up



Due to the growing interest in membrane transporters and pumps as drug targets there is an increasing demand for reliable high throughput measuring systems. **Here we designed and validated an automated full parallel 96 well based platform**, employing SSM base electrophysiology. The method is not only unique in its target and assay flexibility, it's also providing high quality data and it's label- and radioactivity free – Properties ideal for a high throughput system.

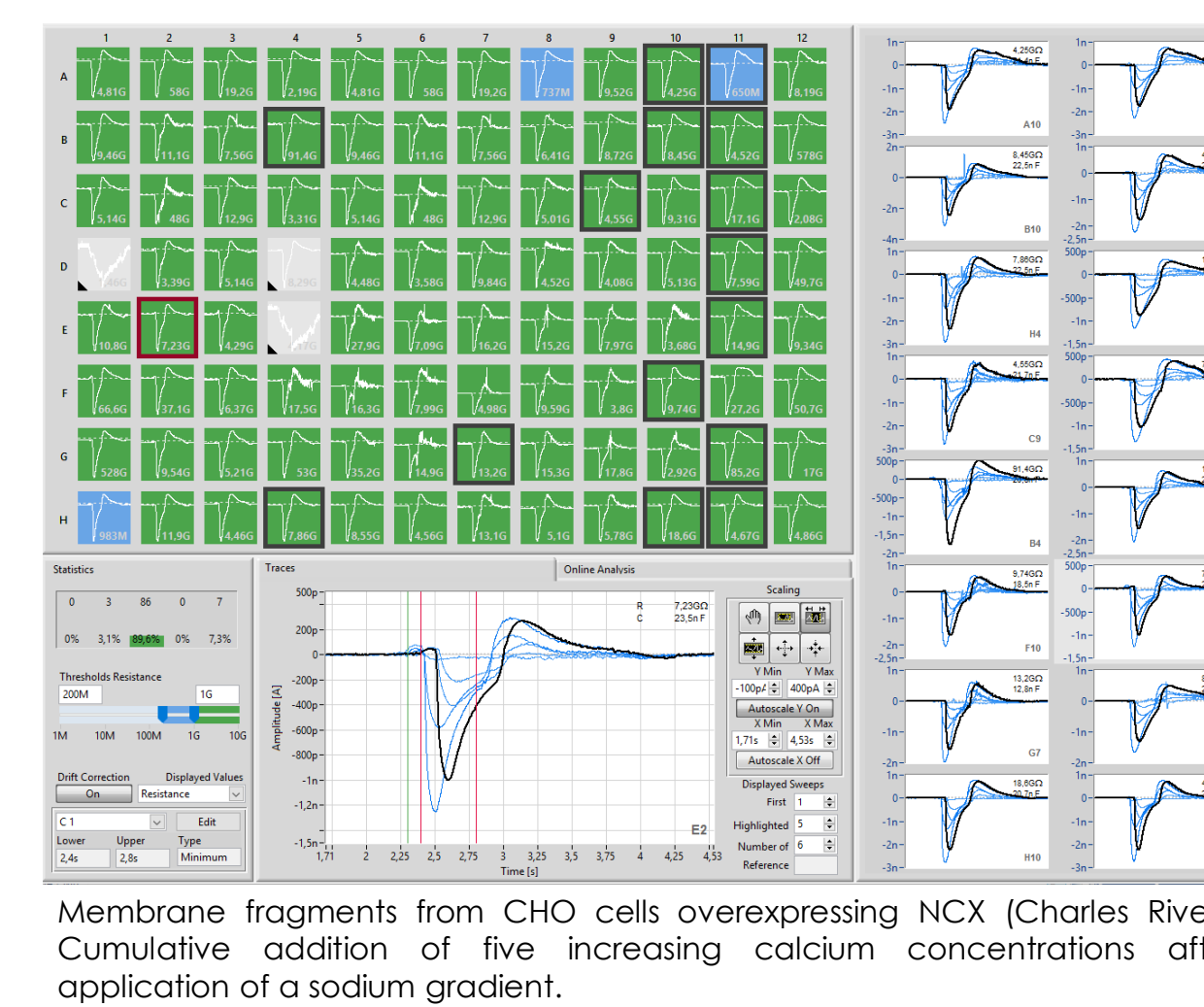


A specialized recording chamber incorporating a 96 well sensor plate, a 96 channel low noise amplifier and respective control electronics was included into a Cybio Felix liquid handling robot. Optimized assay workflows and software tools for recording and analysis were developed, enabling the recording of 96 data points in less than 5 minutes. Additionally the preparation of the SSM sensor plates can be performed by the platform itself.

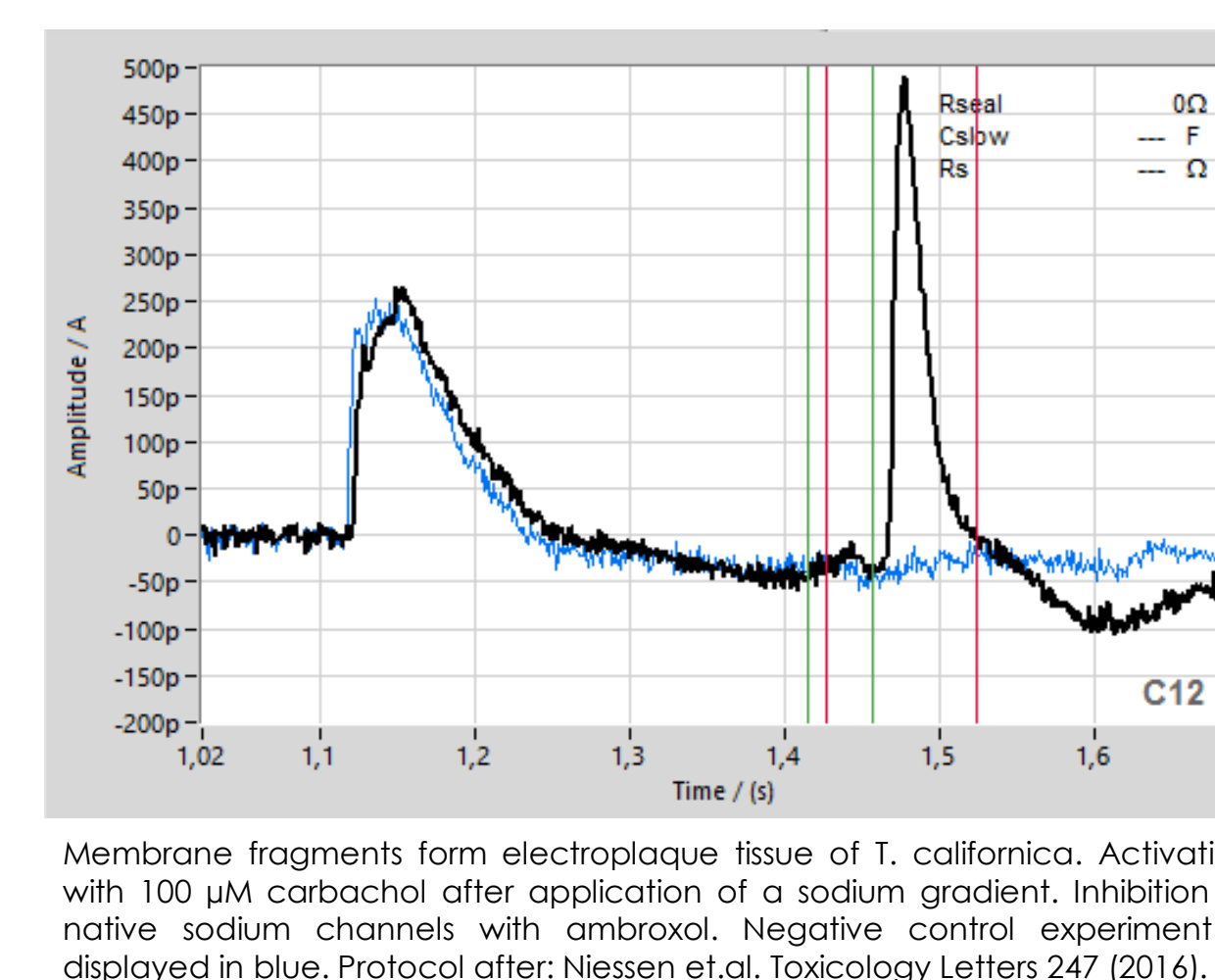


Evaluation of Target Scope

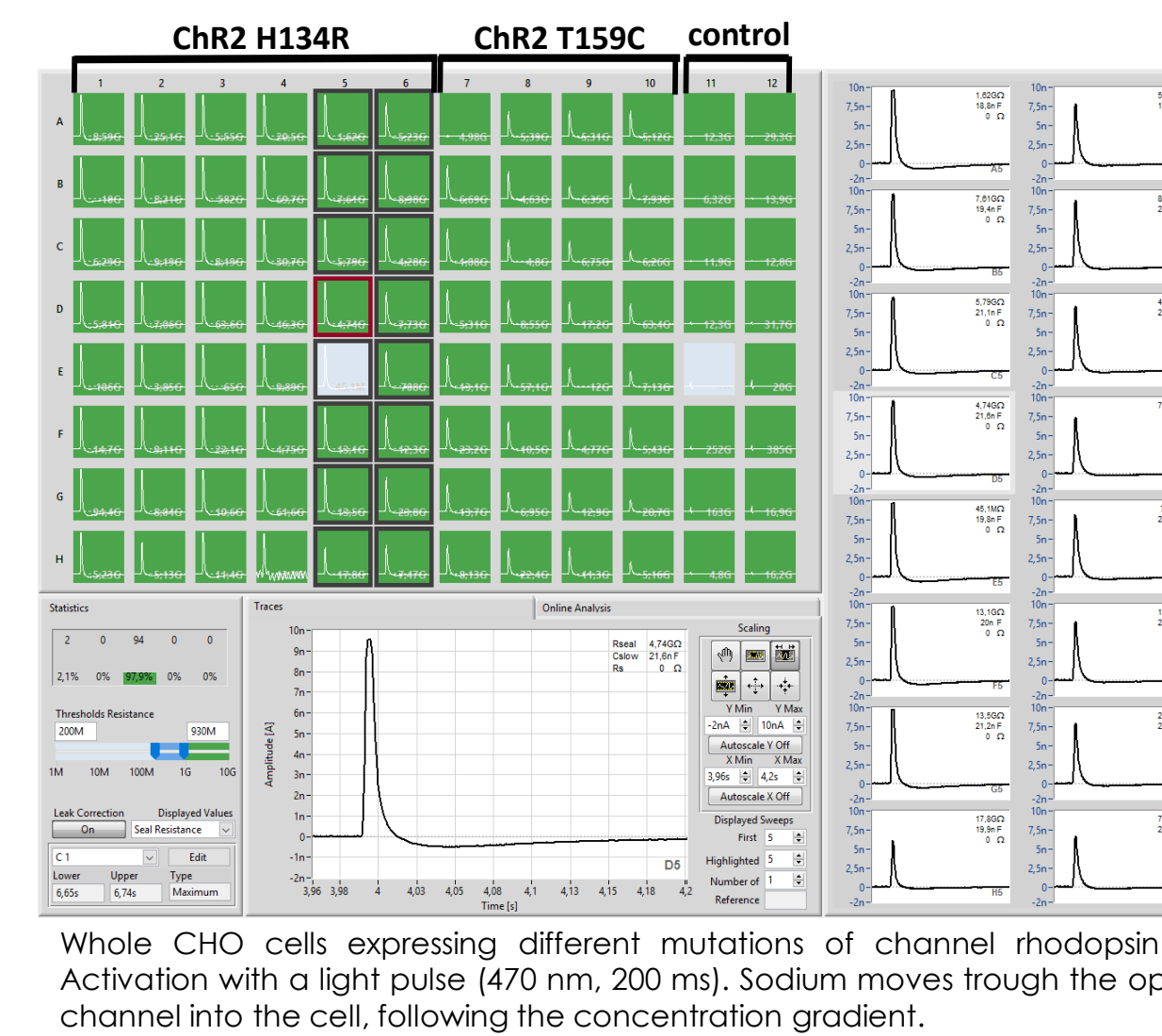
Sodium/Calcium Exchanger (NCX1)



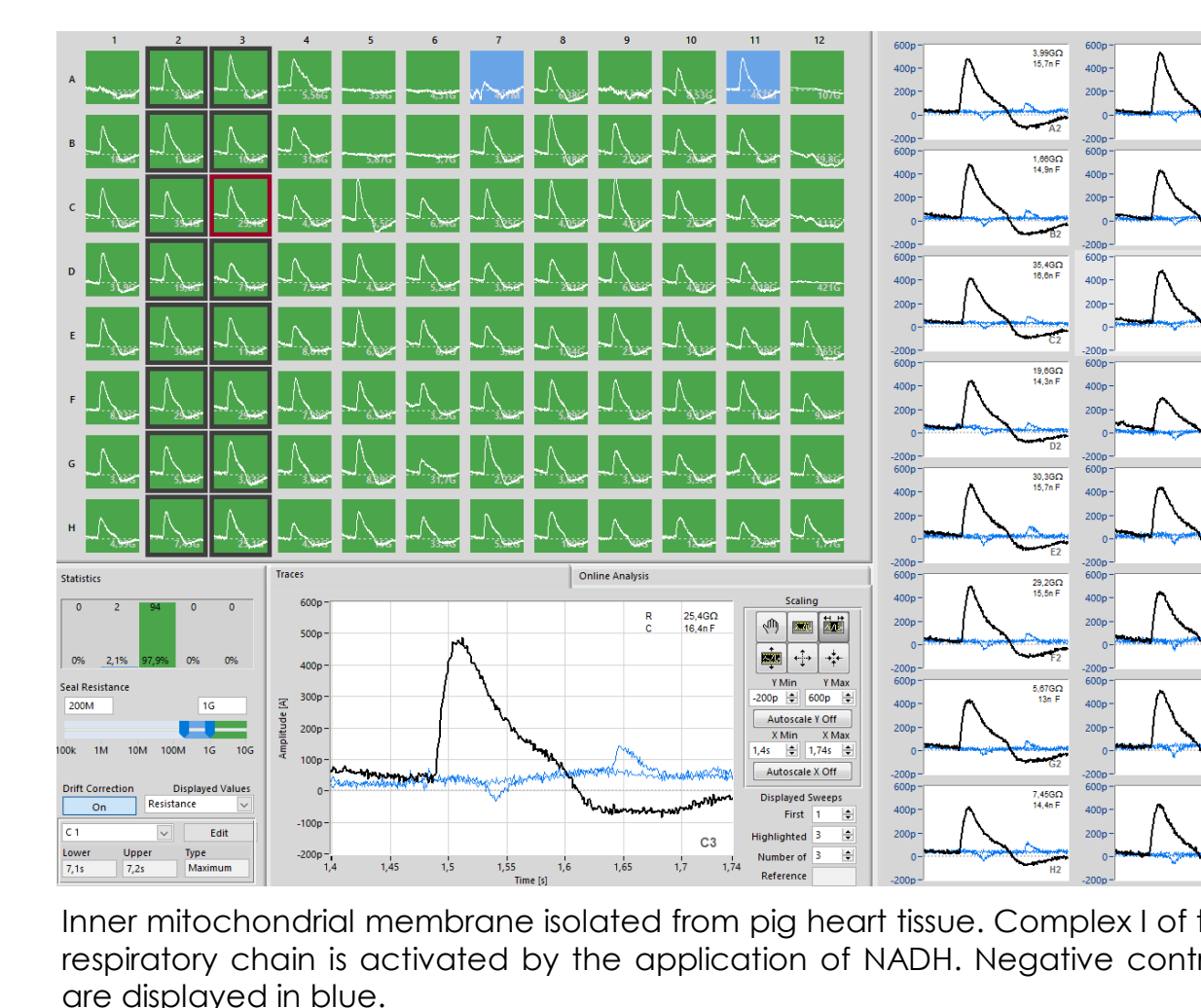
Nicotinic acetylcholine receptor



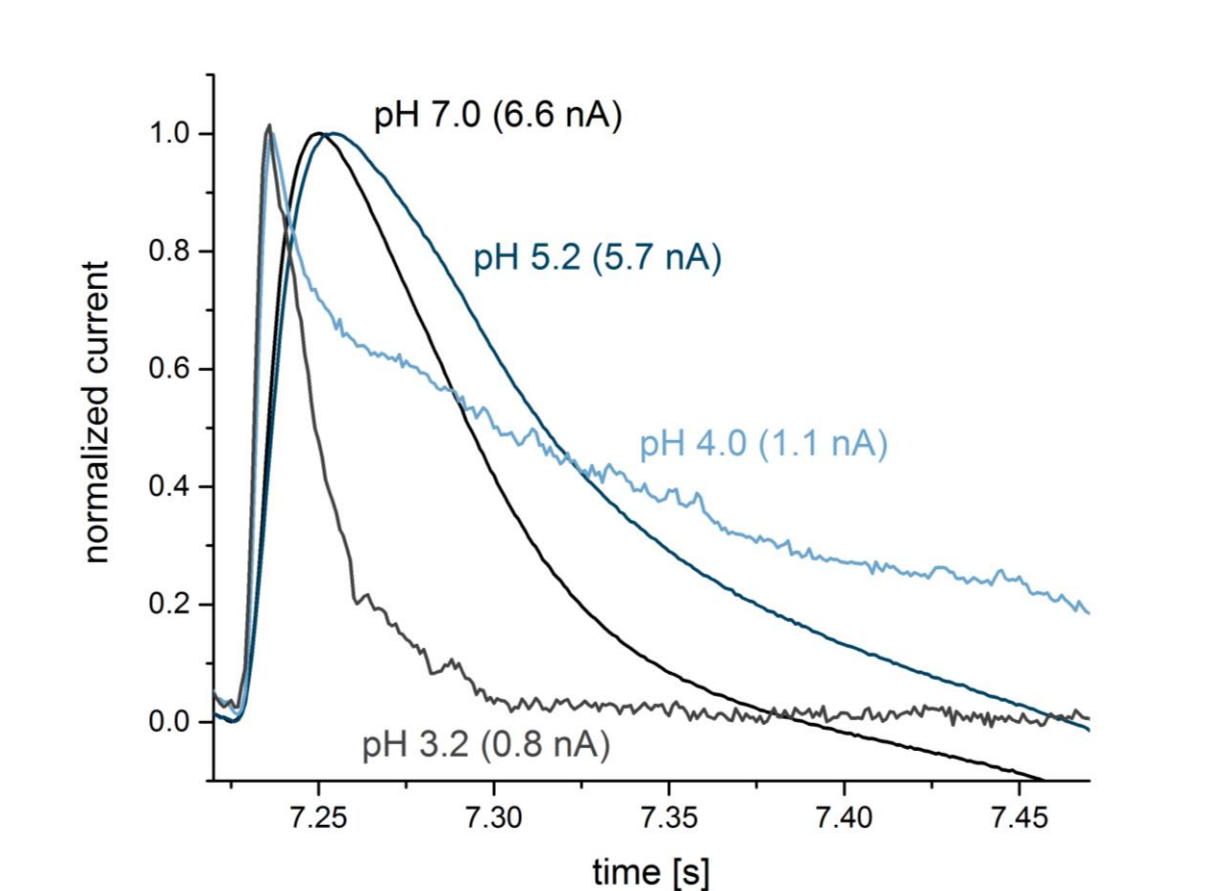
Channel rhodopsin 2



Respiratory chain complex I (enzymatic proton pump)



bacterial sugar transporter (XylE wt)



Mitochondrial F-Type ATPase

