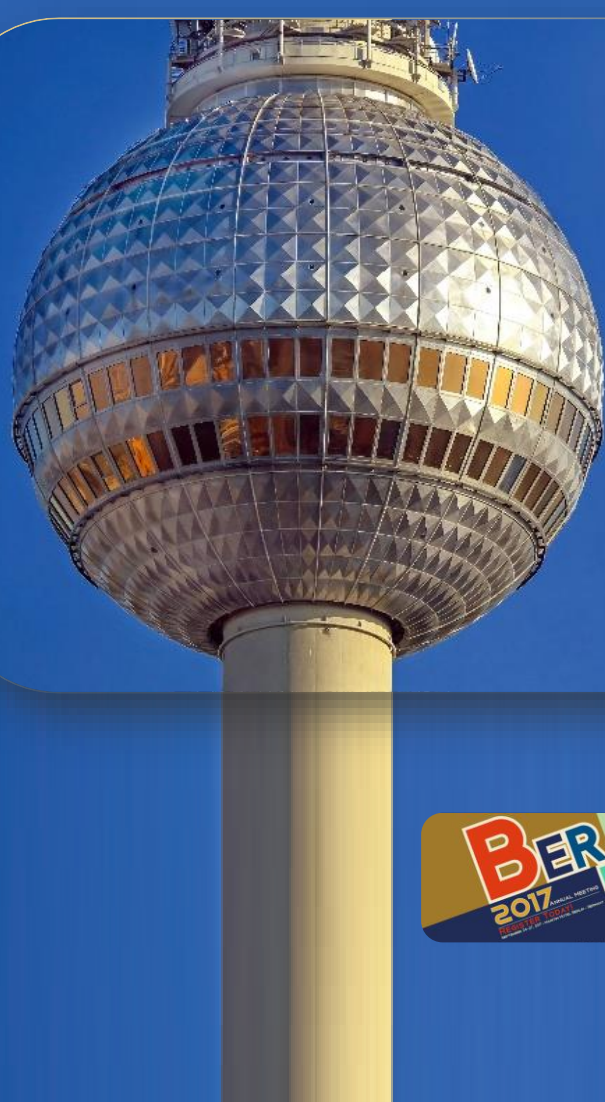




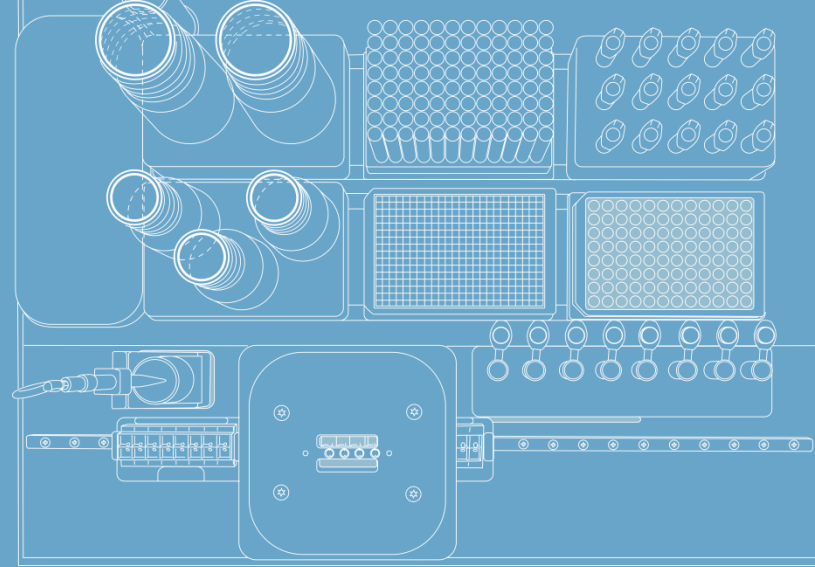
HTS in Cardiac Safety – hosted by Nanion

Satellite symposium during SPS

- Maritim Hotel Berlin
- Sunday, 24th September 2017
- 4:00 – 6:30 PM
- Salon 15 / Paris (1st floor)



Nanon Technologies



HTS in cardiac safety

**Dr. Sonja Stölzle-Feix, Director, Scientific Affairs,
Nanon Technologies**

nan]i[on

Nanion participation in CiPA



- ✓ Part of the leadership team in the HTS ion channel screening subteam:
5 cardiac ion channels recorded on automated patch clamp devices
(Patchliner & SyncroPatch384 PE and others)
- ✓ Participant in the myocyte phase II study:
hiPS cardiomyocytes on impedance/EFP system: the CardioExcyte96

CiPA power tools



Agenda

**SyncroPatch 384 PE & cardiac
ion channels in cell lines and iPS CM**

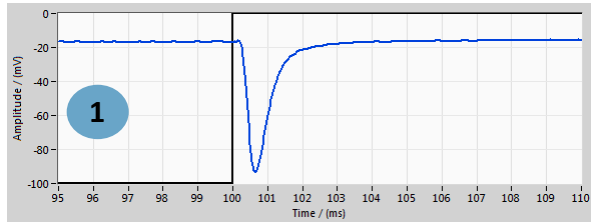
CardioExcyte 96 & cardiac
pharmacology in iPS CM

Summary

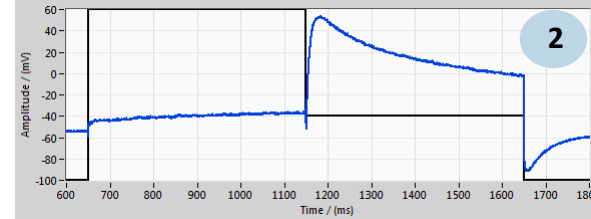


Ion Channels of the CIPA Initiative

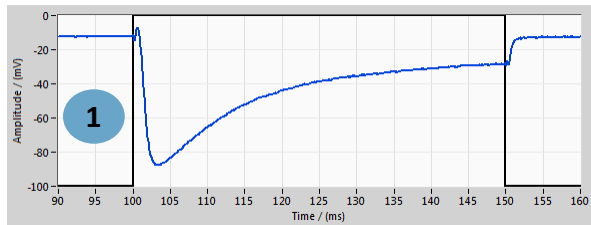
$\text{Na}_v1.5$ („ I_{Na} “)



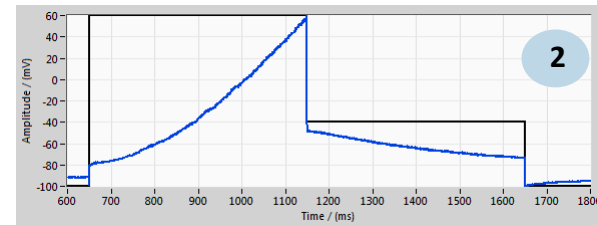
$\text{K}_v11.1$ (hERG – „ I_{Kr} “)



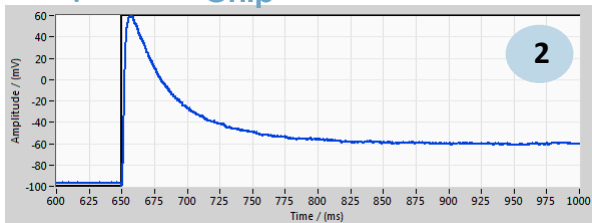
$\text{Ca}_v1.2/\beta2/\alpha2\delta$ („ I_{Ca} , I “)



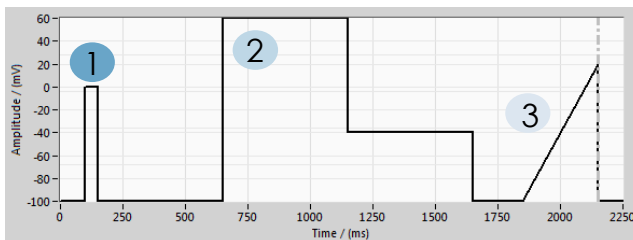
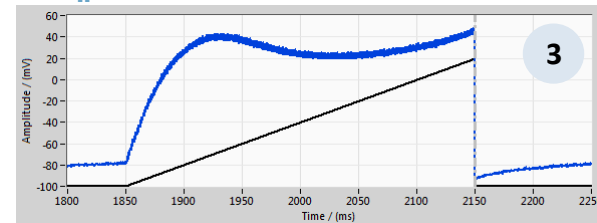
$\text{K}_v7.1\text{-KCNE1}$ („ I_{Ks} “)



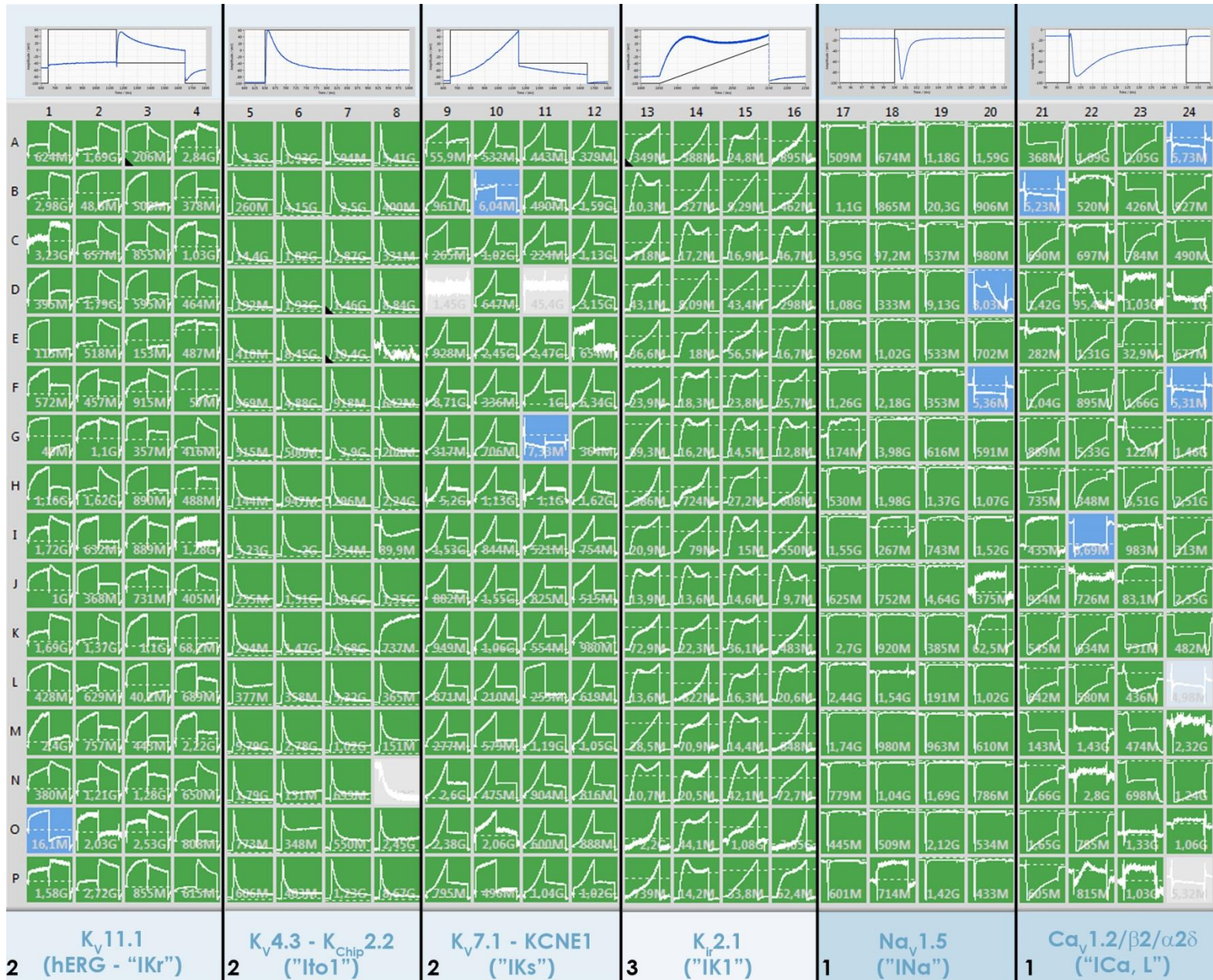
$\text{K}_v4.3\text{-K}_{\text{Chip}}2.2$ („ I_{to} “)



$\text{K}_{\text{ir}}2.1$ („ I_{K1} “)



Ion Channels of the CIPA Initiative



Different (iPS CM) cells on one Plate

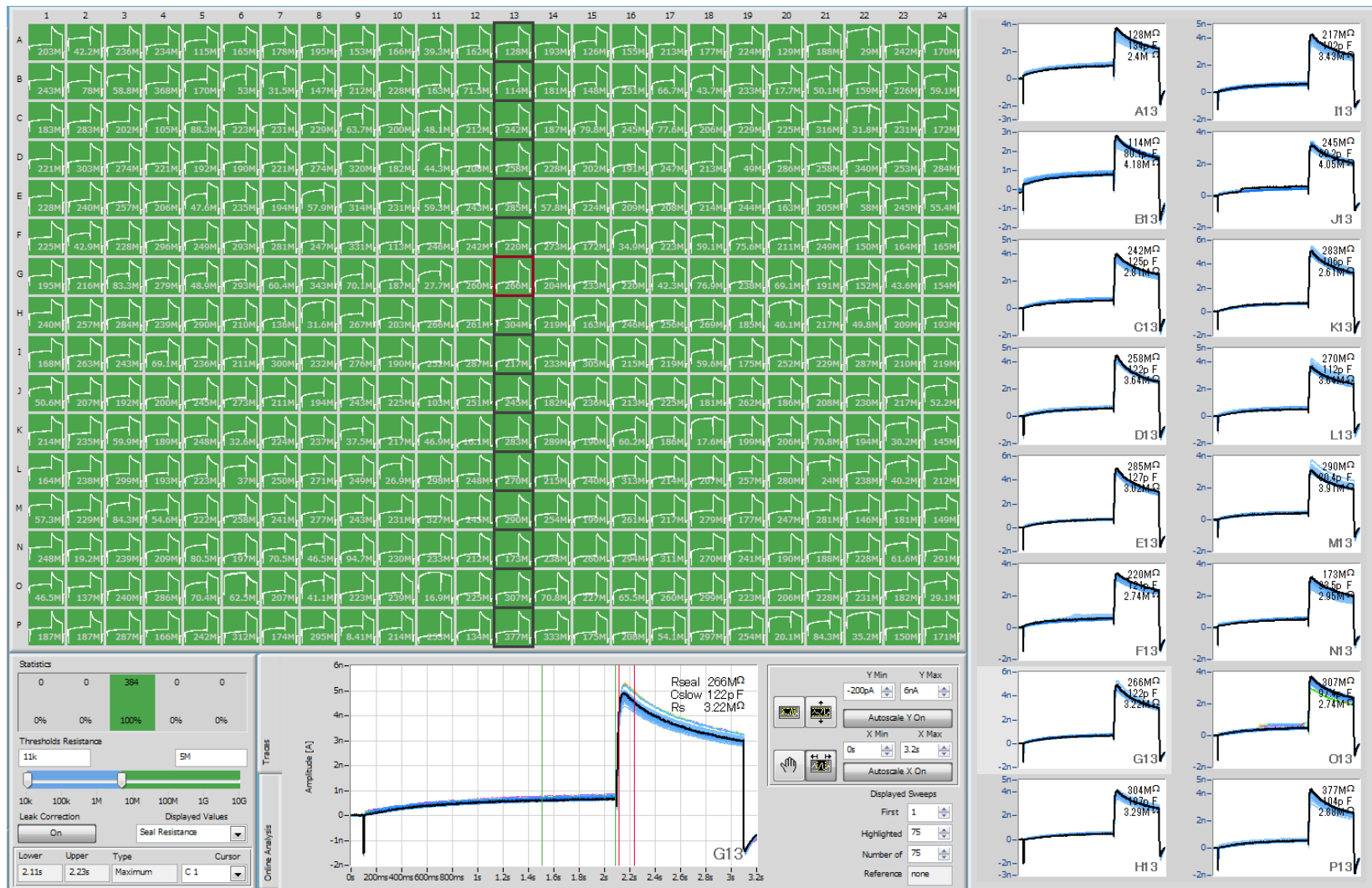
iCell²

Cor.4U

CHO Nav1.7

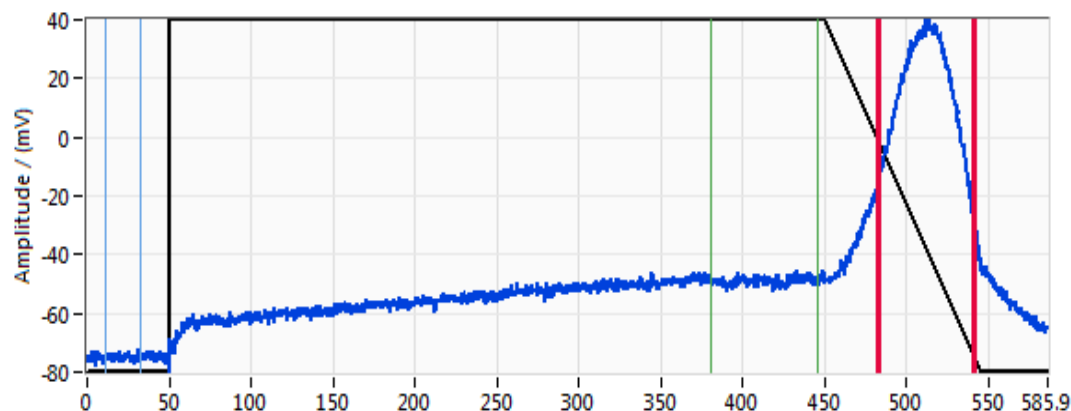


CHO hERG Current Stability

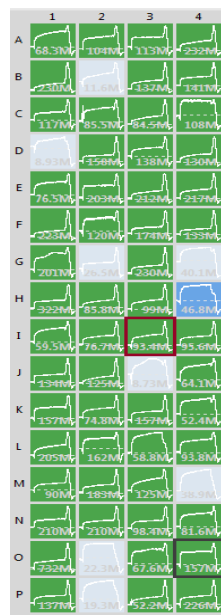


Overlay of the Traces for more than 20 minutes

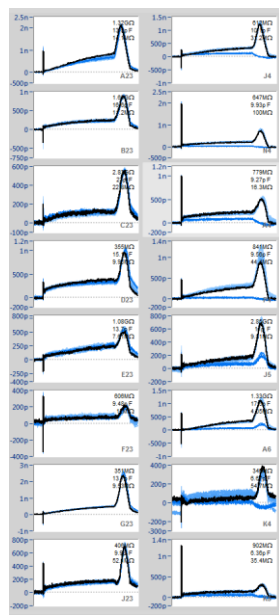
CiPA Step-Ramp hERG protocol



hERG step-ramp protocol: depolarization for 400 ms at 40 mV followed by a 100 ms ramp segment where the hERG tail current is measured. The protocol runs at 0.2 Hz for 5 min to establish baseline and 5 min for compound incubation.



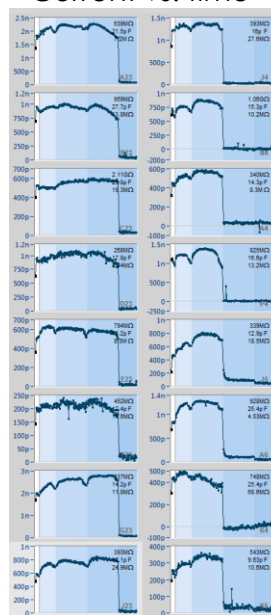
Raw data traces



Neg.

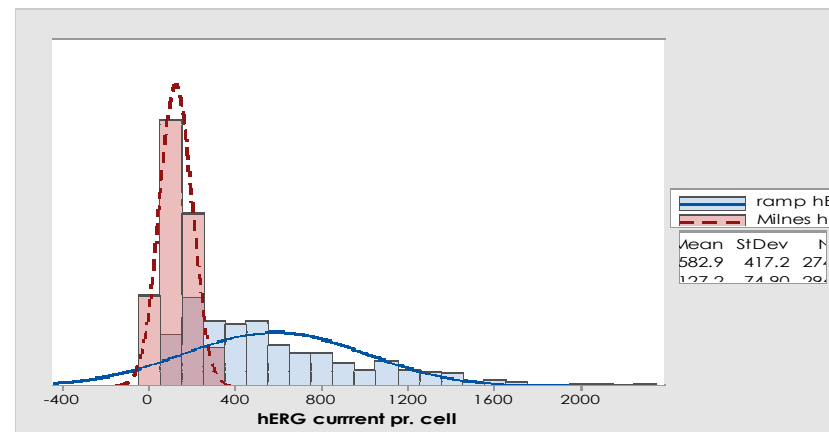
Pos.

Current vs. time

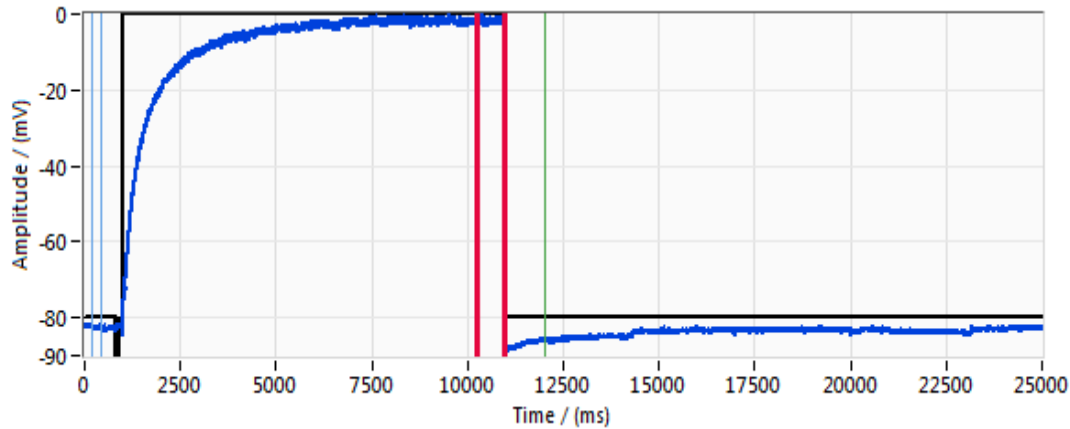


Neg.

Pos.

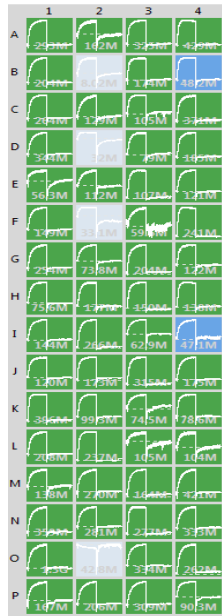


CiPA Milnes hERG protocol

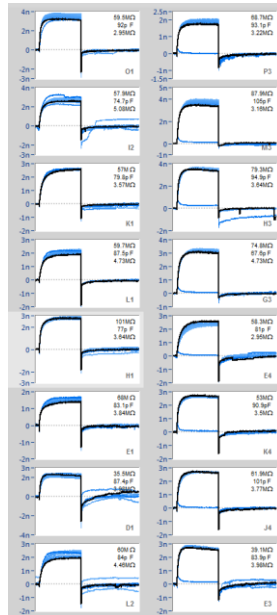


hERG experiments are also done with the Milnes protocol in order to collect data for the in-silico modeling group.

The protocol is executed 10 times every 30 seconds.



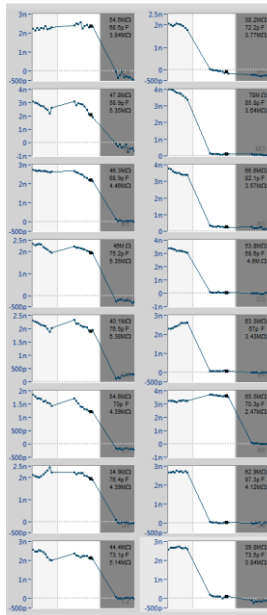
Raw data traces



Neg.

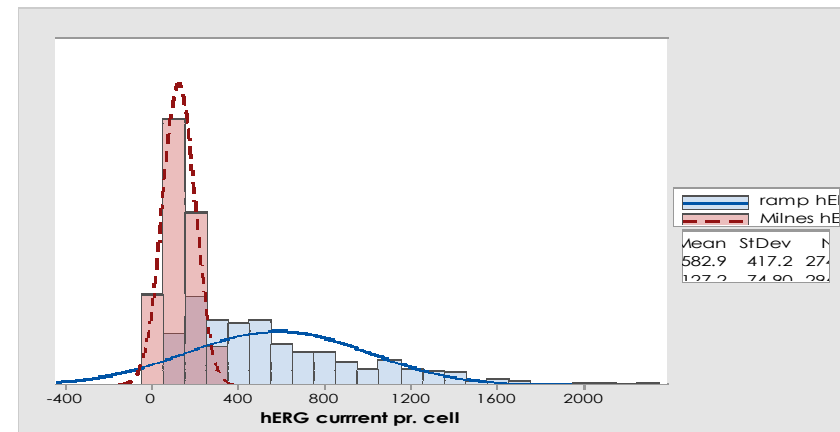
Pos.

Current vs. time



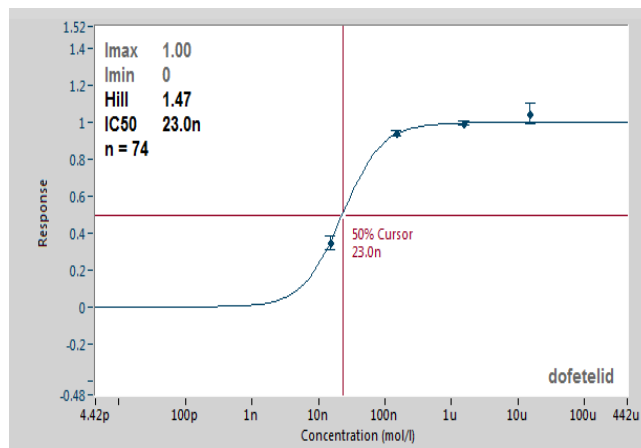
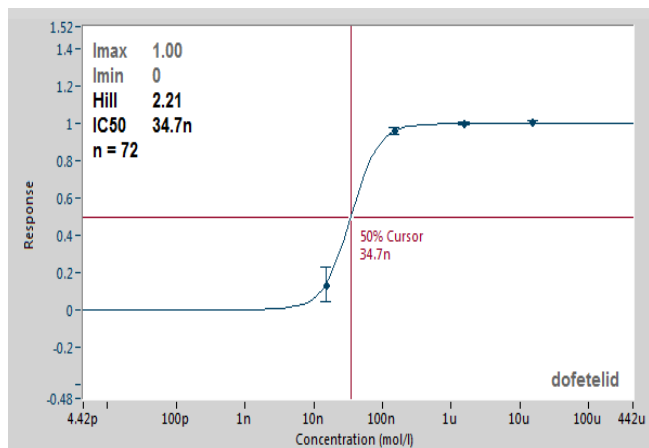
Neg.

Pos.

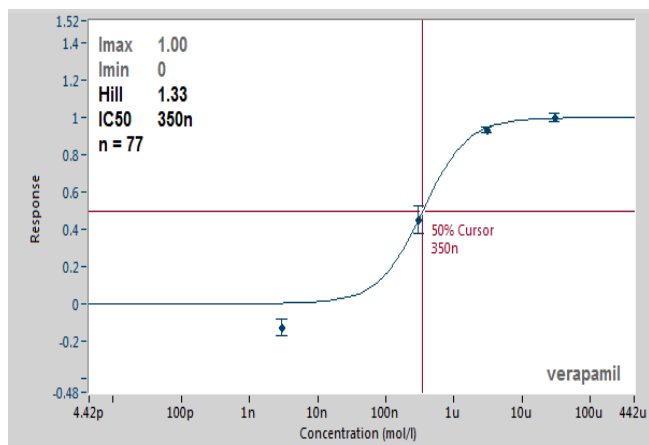


Pharmacology:

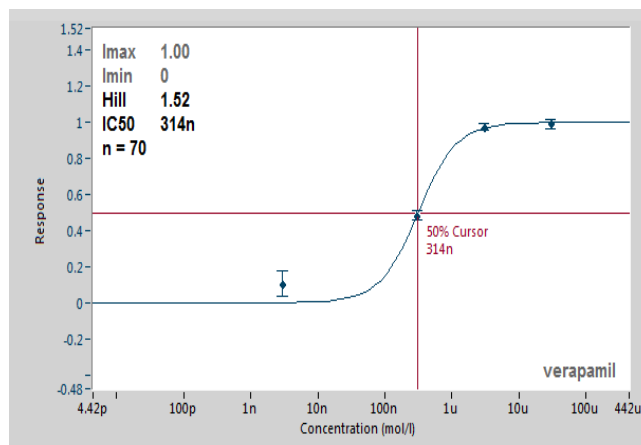
Milnes vs . Step-Ramp hERG protocol



The Milnes and the ramp protocol were compared in terms of IC₅₀'s of Verapamil and Dofetilide.



Milnes

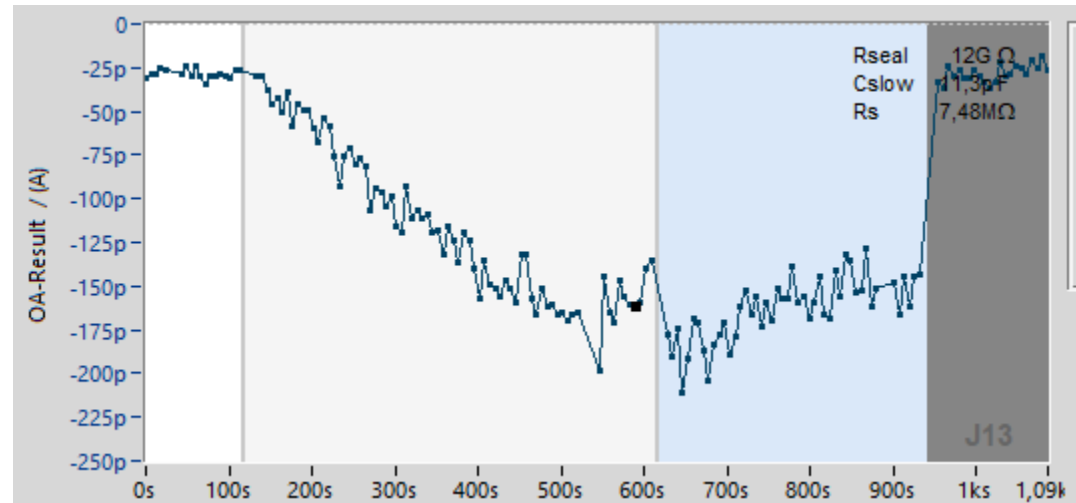
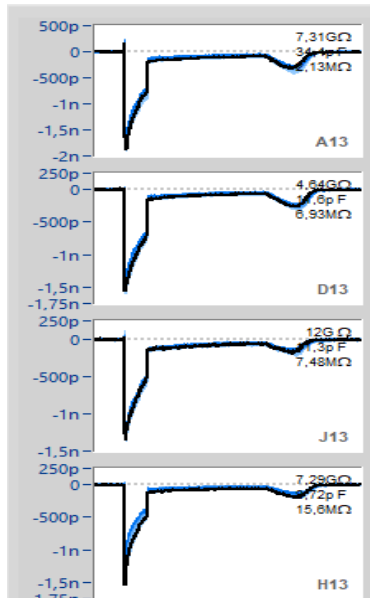


Step-Ramp

The IC₅₀'s were found to be comparable between the two methods.

CHO hNav1.5 Late Current

Nav1.5 Late – Activation by ATX-II - Current Over Time



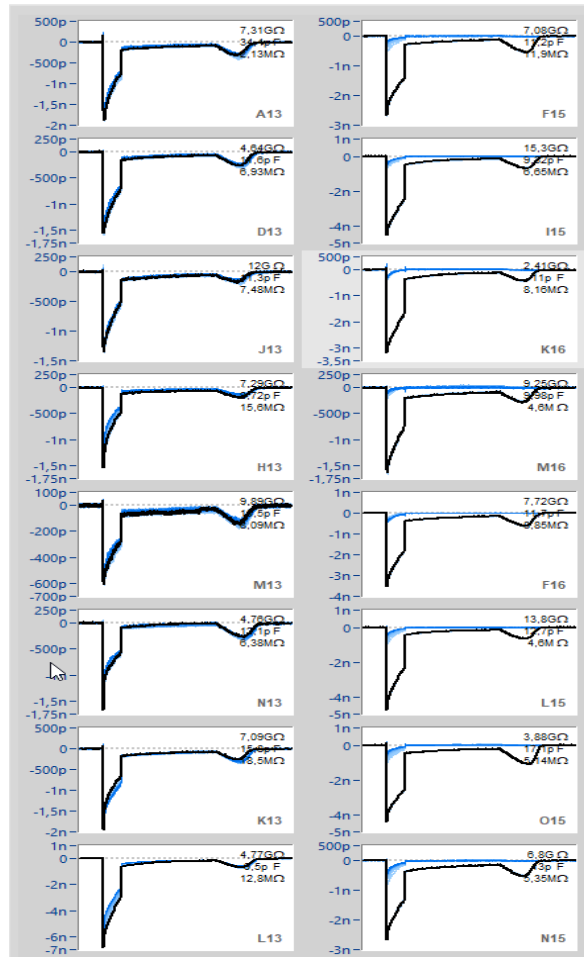
Reference phase
ATX-II Activation

Compound phase
(here DMSO only)

Full Block phase

Nav1.5 Late Raw Traces and Current Over Time

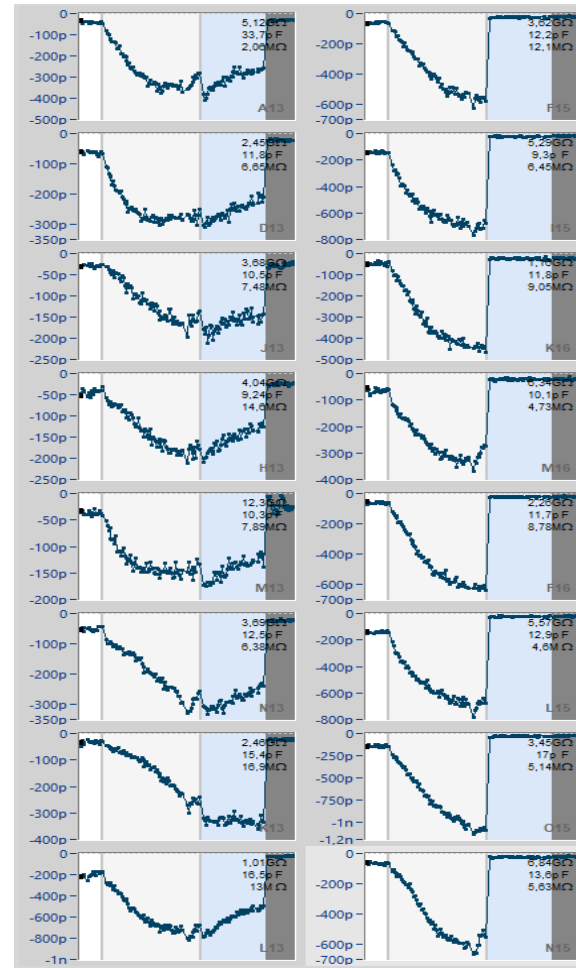
Raw Traces after ATX-II Application



neg. control
(DMSO only)

pos. control
(50 μ M Tetracaine)

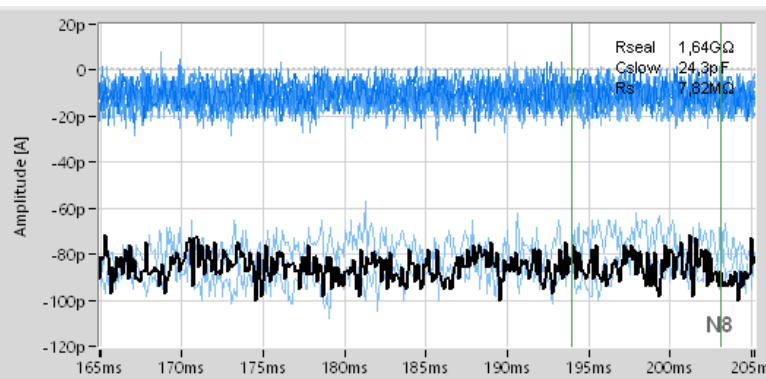
Current Over Time



neg. control
(DMSO only)

pos. control
(50 μ M Tetracaine)

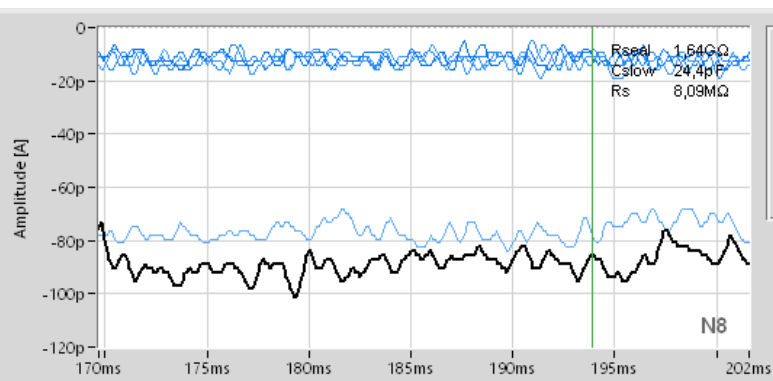
CHO hNav1.5 Late Current



**10 kHz
Sampling**

**Inhibition of the late Nav1.5
current by 500 μ M Tetracaine.**

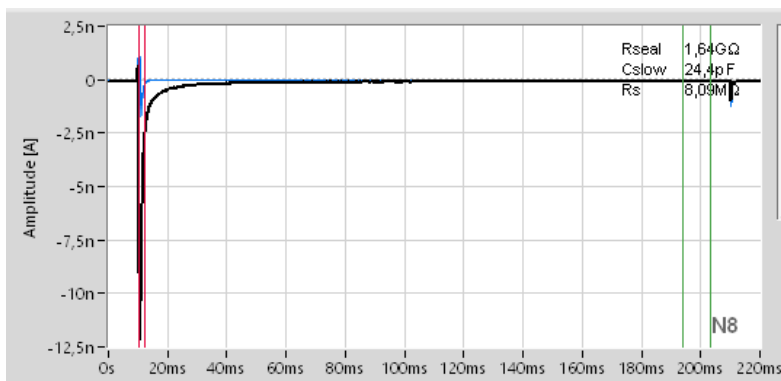
**No leak subtraction was
applied.**



**1 kHz
Butterworth
Filter**

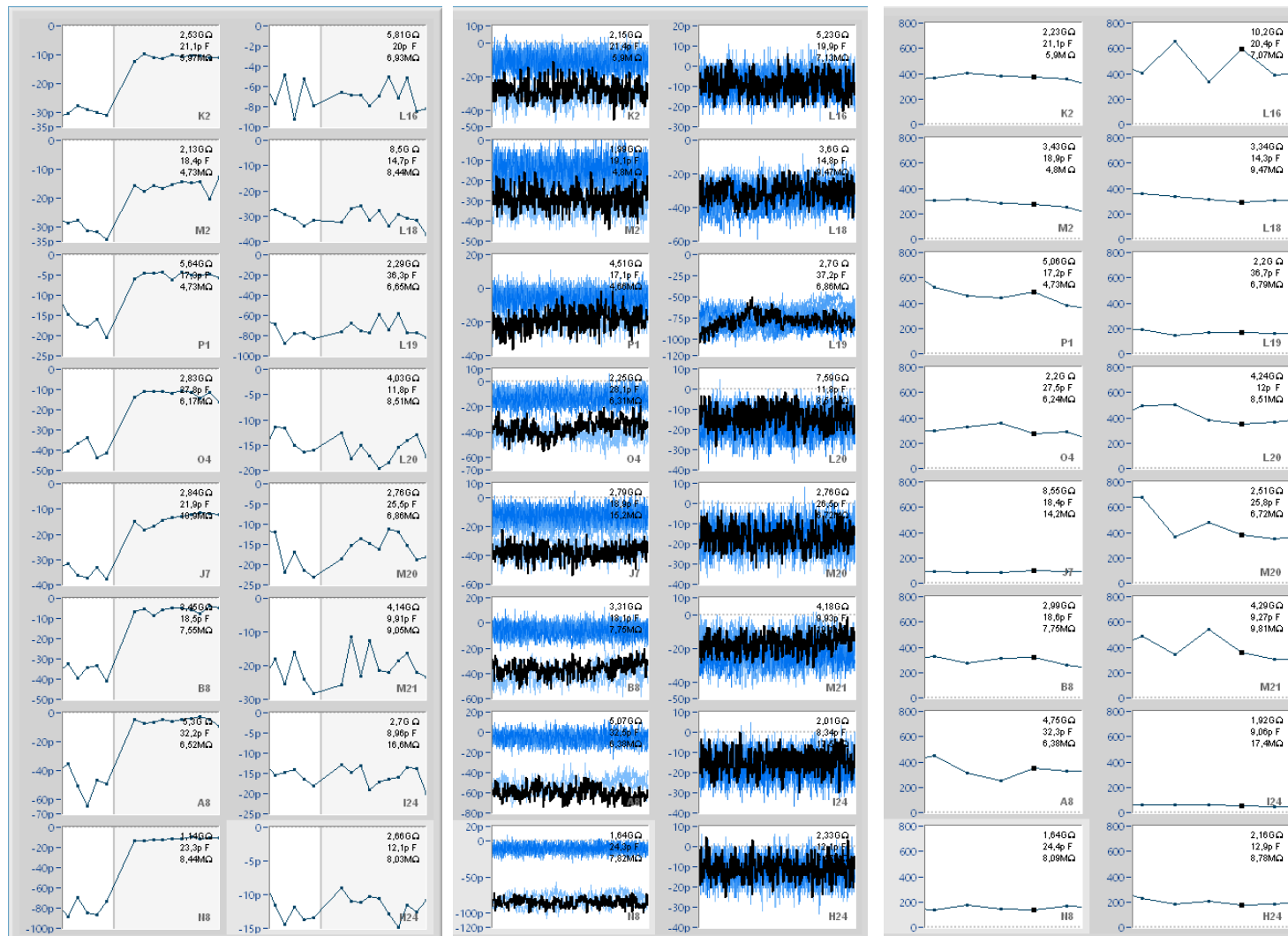
**No pharmacological tools (ATX-
II) were used to increase the
late current.**

**Whole-cell recording
single hole chip**



**Complete
Trace
10 kHz**

CHO hNav1.5 Late Current



Inhibition

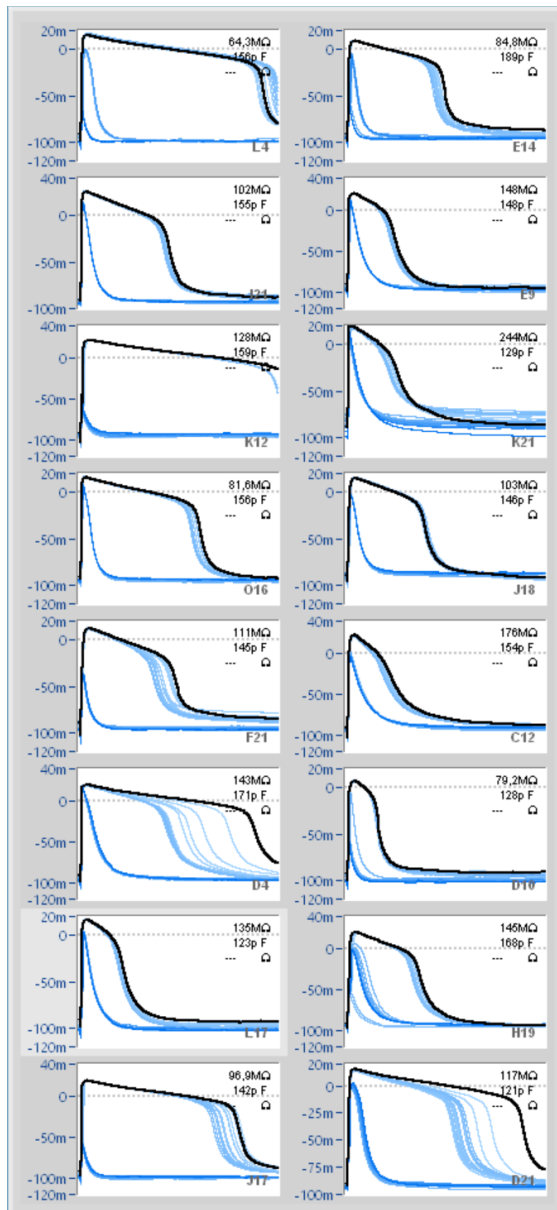
Control

Inhibition

Control

Ratio Peak/Late Current

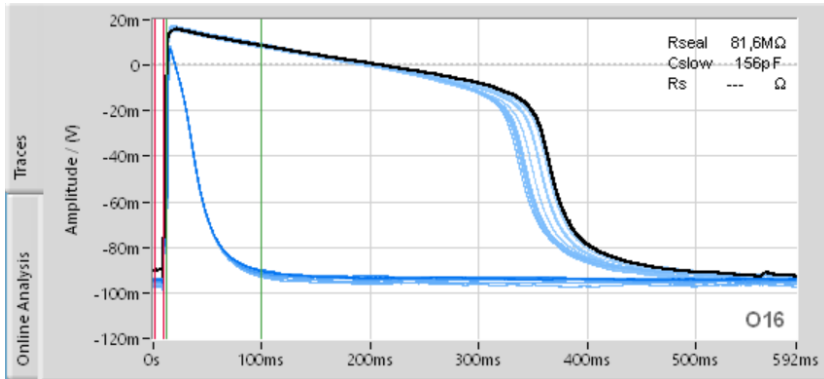
Current Clamp: iPS derived Cardiomyocytes Cor.4U



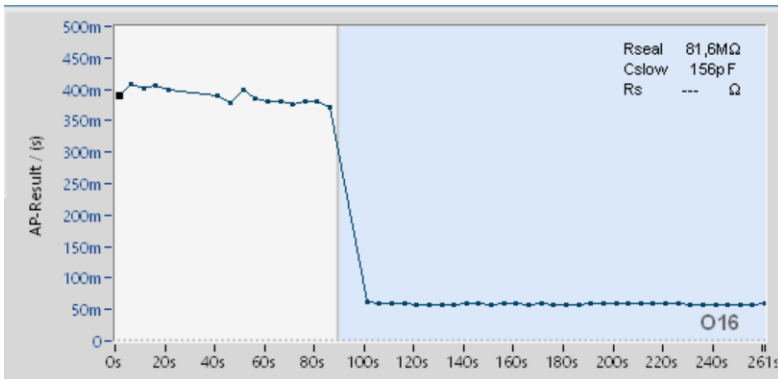
The AP was recorded in 5 mM Barium. Different shapes and AP lengths can be observed.

5 μ M Nifedipine was applied for Calcium channel inhibition. An immediate shortening of the APD can be observed.

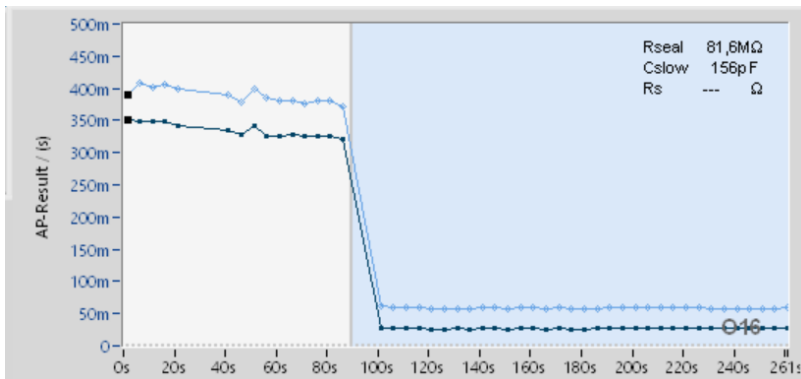
APD shortening by Nifedipine



The AP was recorded in 5 mM Barium.
5 μ M Nifedipine was applied for Calcium channel inhibition.

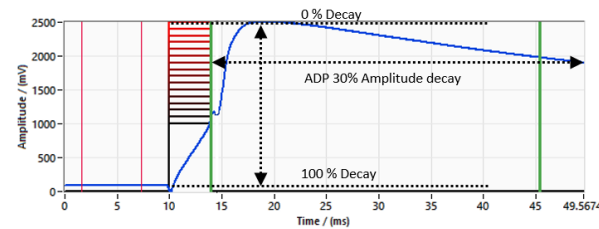
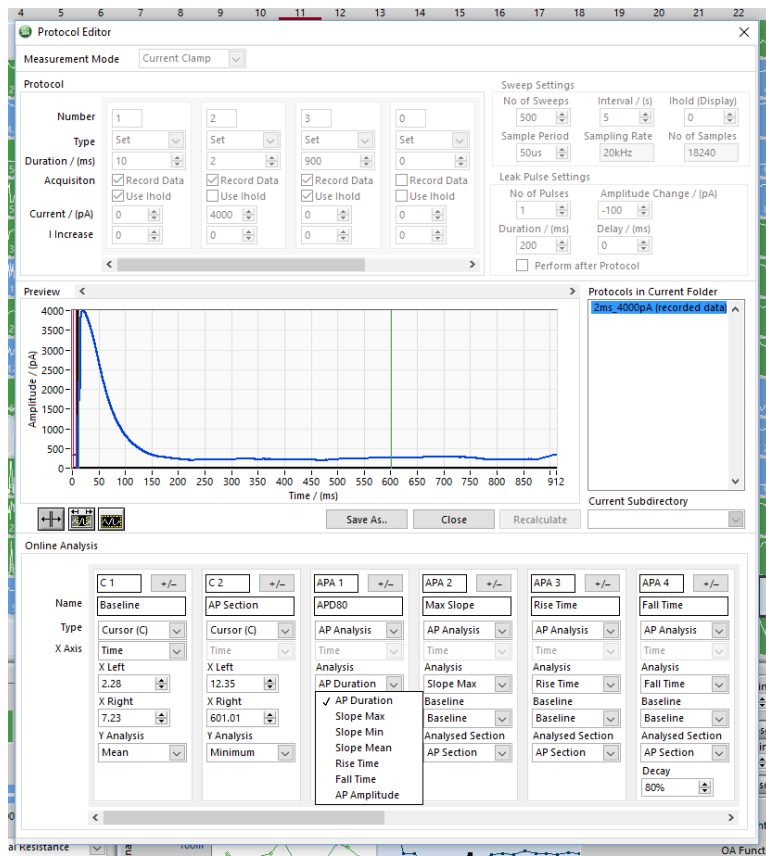


Online Analysis of the same cell.
The APD90 was calculated before and after the inhibition of 5 μ M.



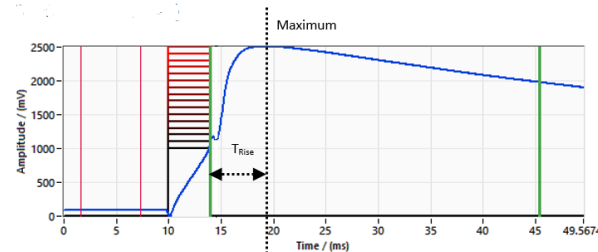
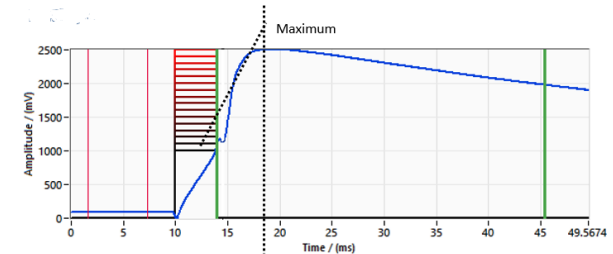
Online Analysis of the same cell.
Comparing APD90 and APD50

Analysis Functions for Current Clamp Measurements



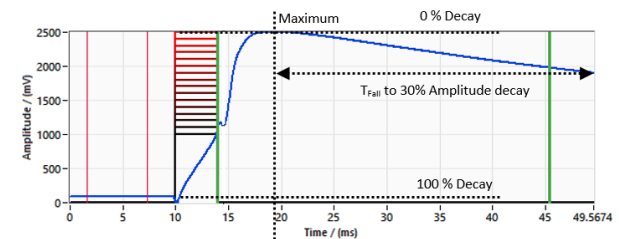
AP Duration

Slope

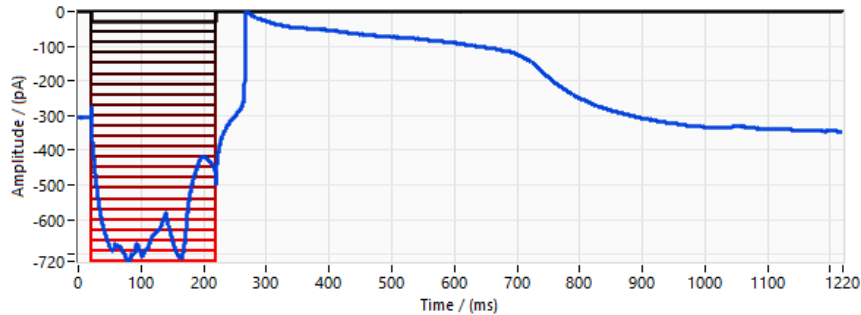


Rise Time

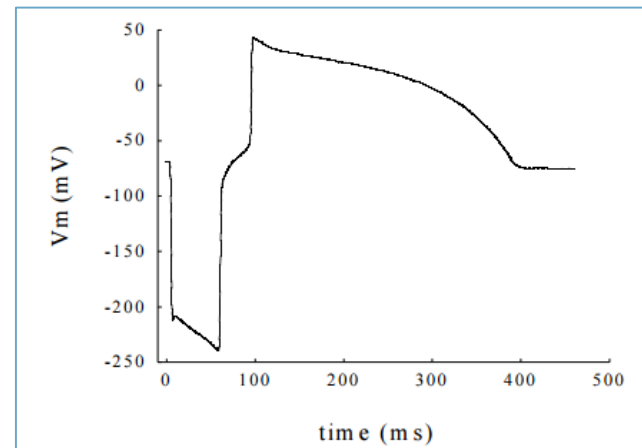
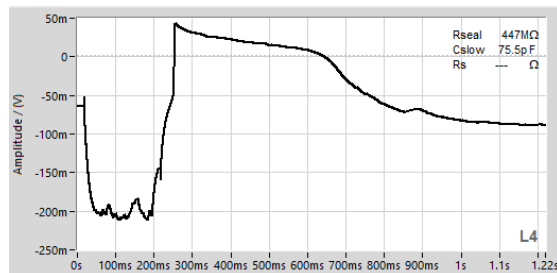
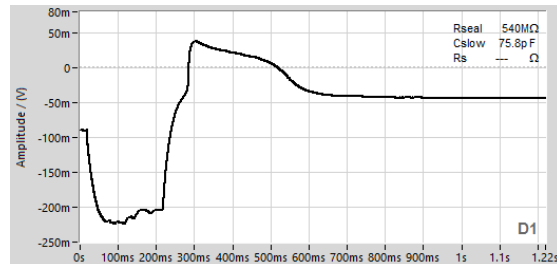
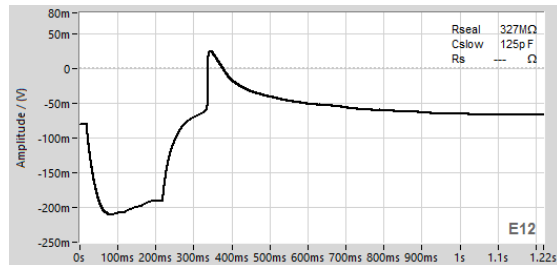
Fall Time



Current Clamp: Anode Break Stimulation



„Active cellular properties (*HCN* channels, *Ik1* supporting) explain anode break stimulation in cardiac cells.“



Anodal break in Guinea pig
Ranjan et al. 1998

Patchliner and it's new features

Hardware

- External & internal exchange
- Temperature control
- Voltage & current clamp + dynamic clamp **New!**
- Single- & multihole chips, produced in-house
- Rseries compensation
- Cooled cel hotel **New!**

Applications

- Voltage and ligand-gated ion channels
- Whole-cell & perforated patch clamp
- Cell lines, primary cells and stem cells
- Validated for CiPA
- Minimized cell consumption
- Easy & customizable analysis tools



The Patchliner offers high flexibility and sophistication.

Agenda

SyncroPatch 384 PE & cardiac
ion channels in cell lines and iPS CM

**CardioExcyte 96 & cardiac
pharmacology in iPS CM**

Summary



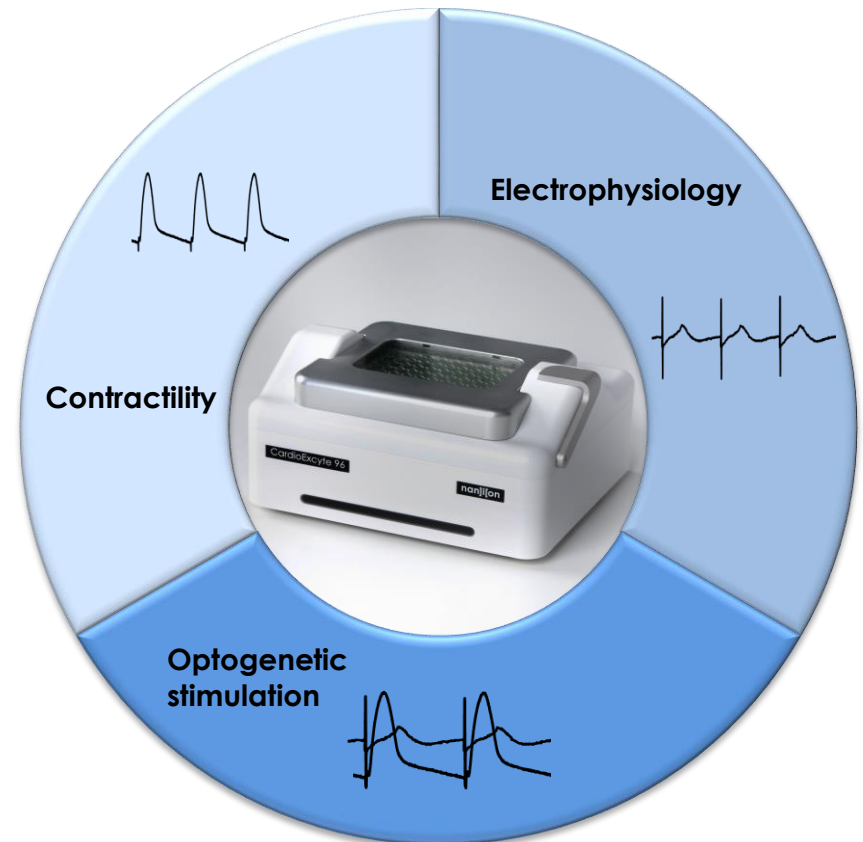
Combined Impedance/Extracellular Field Potential (EFP) assay provides mechanistic electrophysiological endpoints in vitro



✓ Established SOPs for
Impedance/EFP measurements
in frame of the CiPA studies

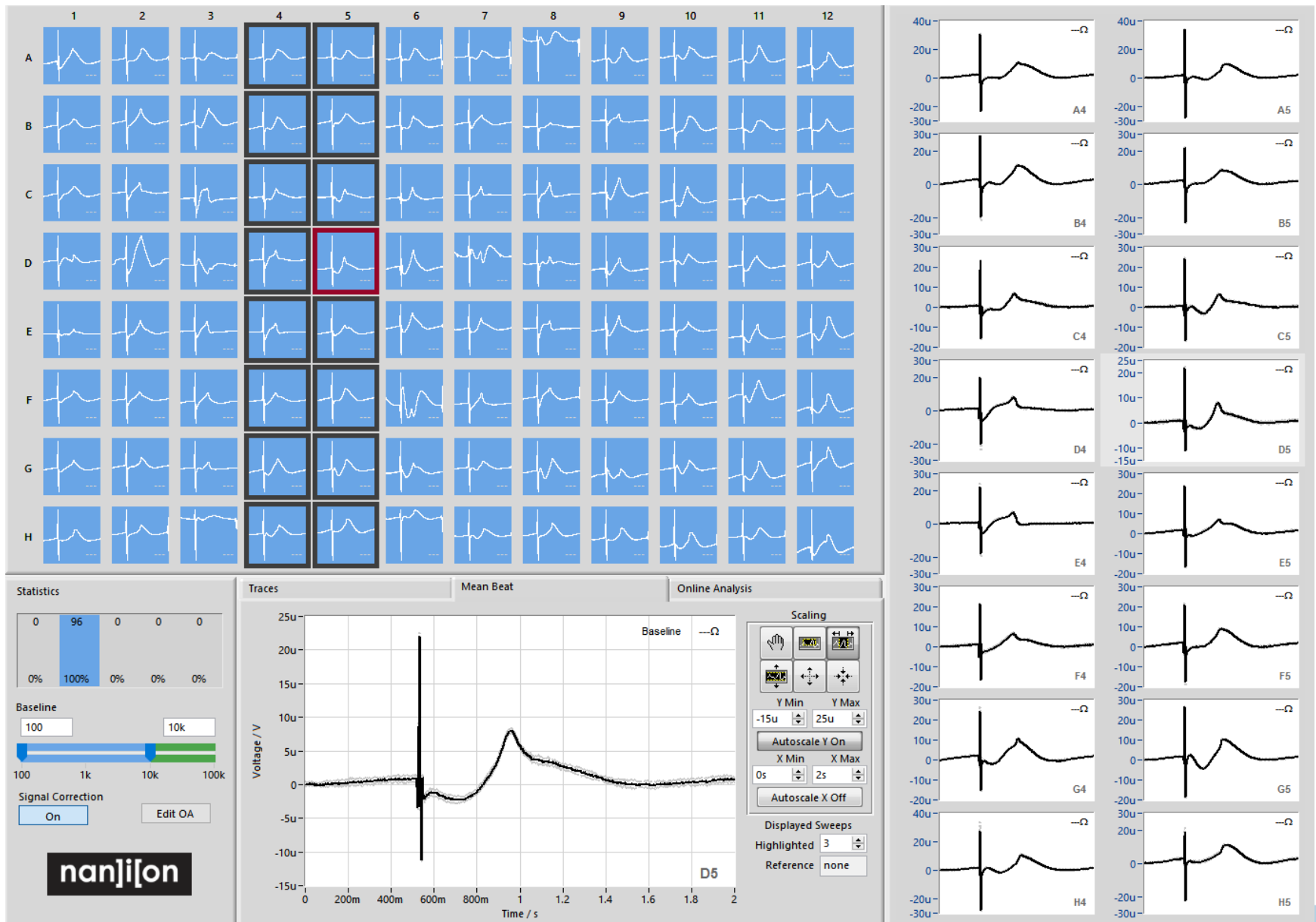


INTEGRA

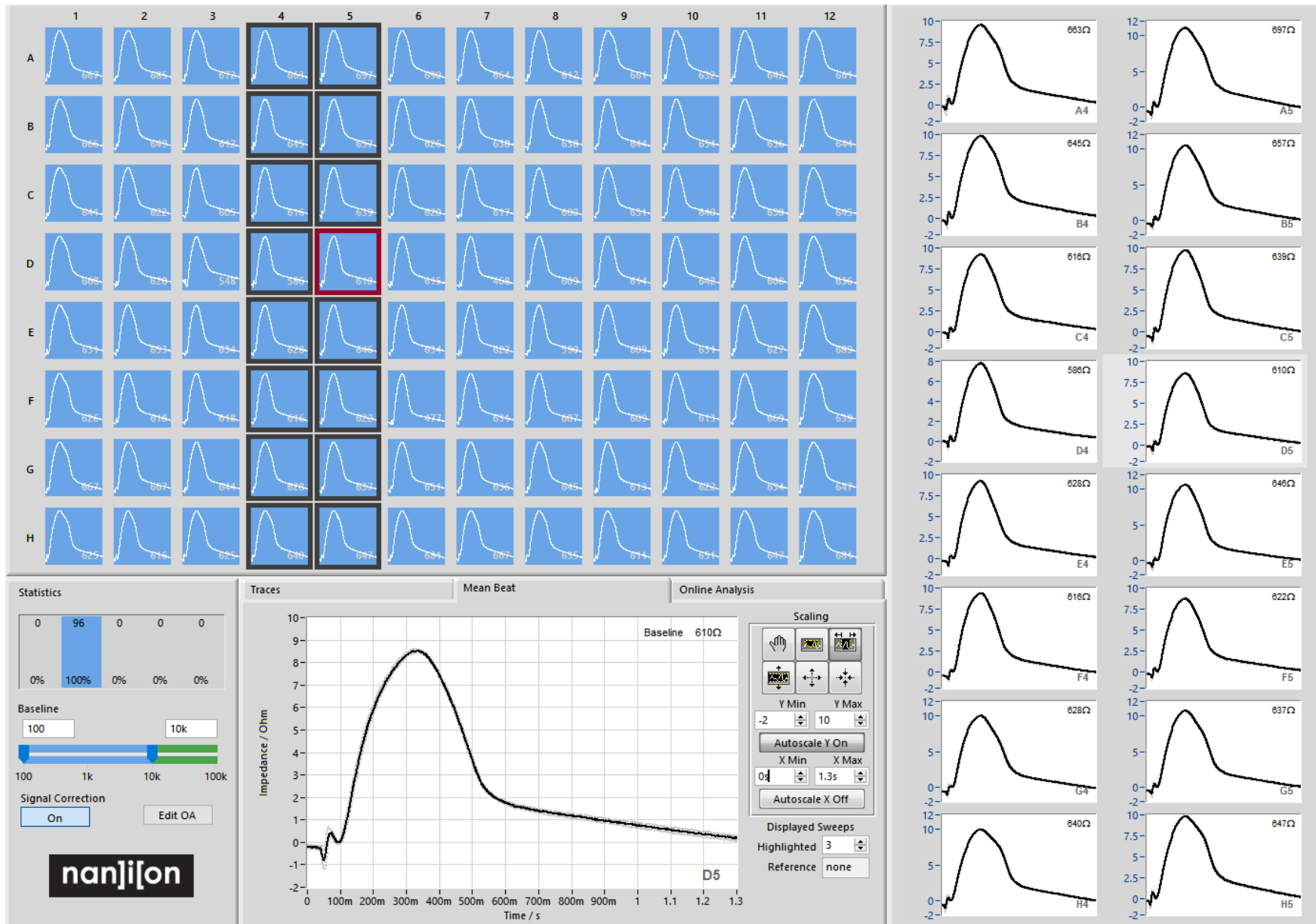


The CardioExcyte 96

- **96 wells** – 96 parallel sensors
- Efficient data analysis
- Automated liquid handling robot for cell seeding, medium exchange

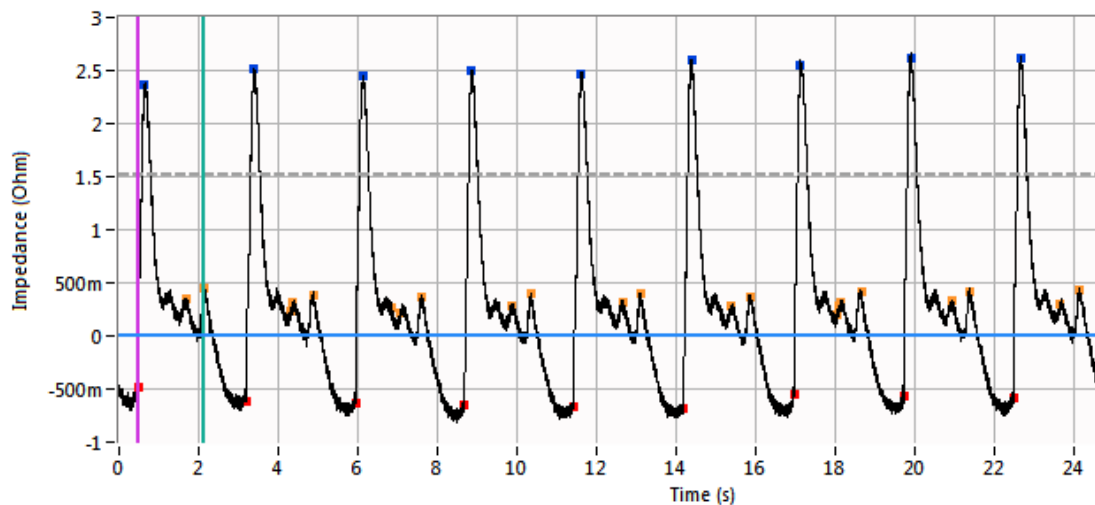


CardioExcyte96 experimental view of mean extracellular field potential signals recorded in stem cell derived cardiomyocytes. Random selection of multiple wells (black squares) allows the display of the impedance recordings on the right. Data from cell type 1

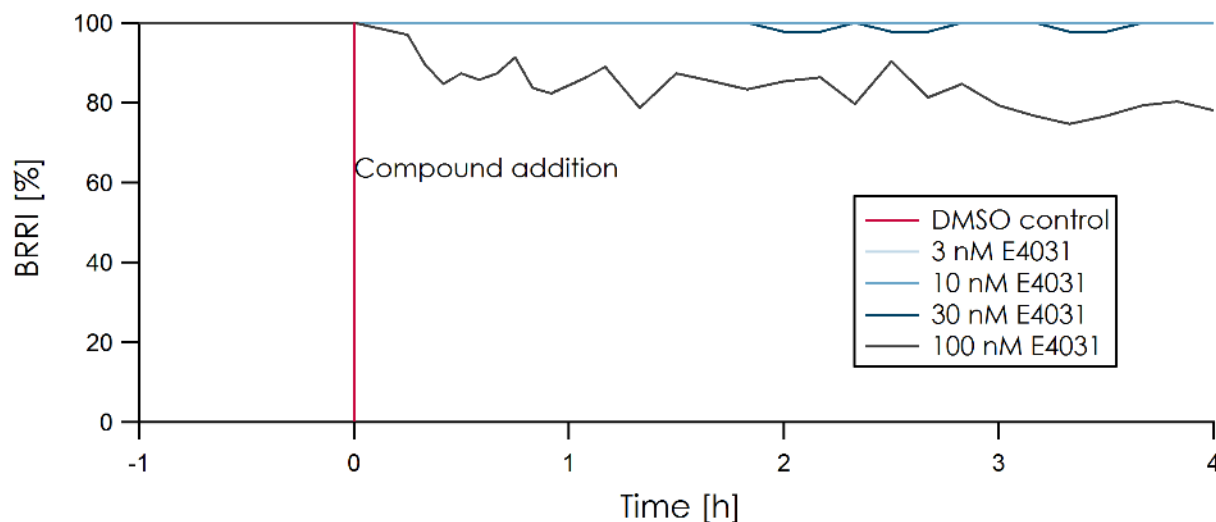


CardioExcyte96 experimental view of mean impedance beat signals recorded in stem cell derived cardiomyocytes. Random selection of multiple wells (black squares) allows the display of the impedance recordings on the right. Data from cell type 1

E4031 induces EADs

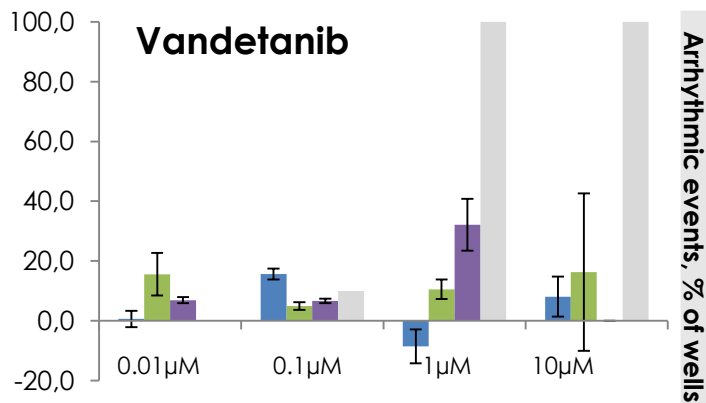
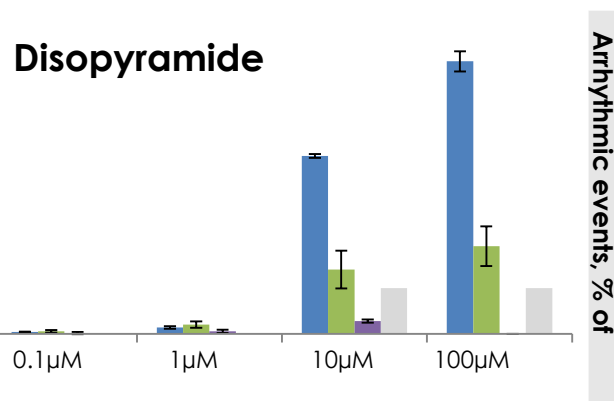
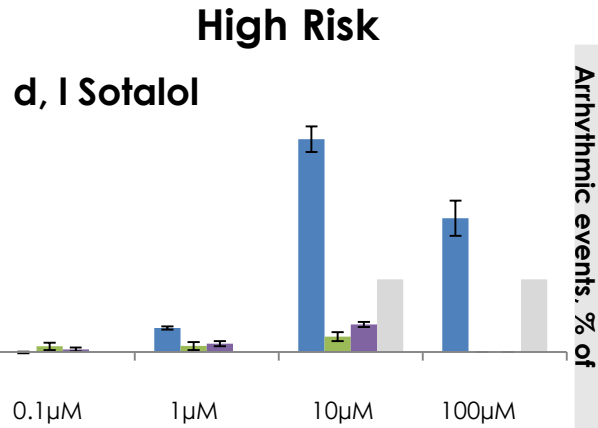


EADs (Early after depolarisations; marked in yellow) detected after 15min (100 nM).

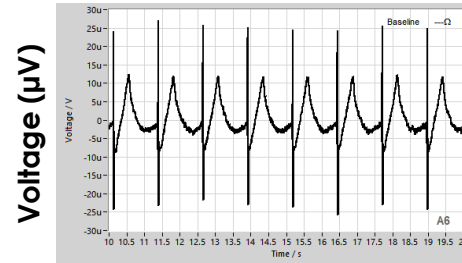
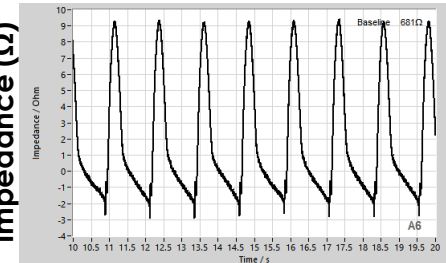


The BRRI dropped down (increasing arrhythmia) to 80 % at 100 nM.

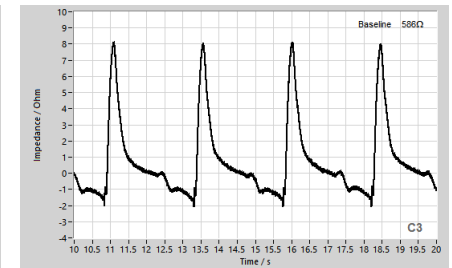
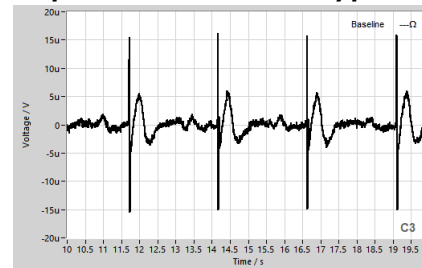
Repolarisation prolongation Repolarisation prolongation Repolarisation prolongation



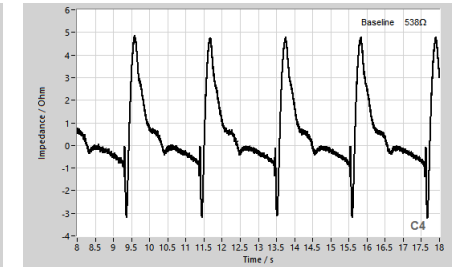
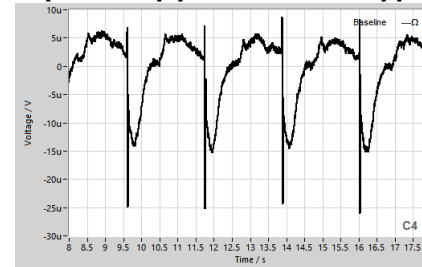
Control, cell type 1

Impedance (Ω)

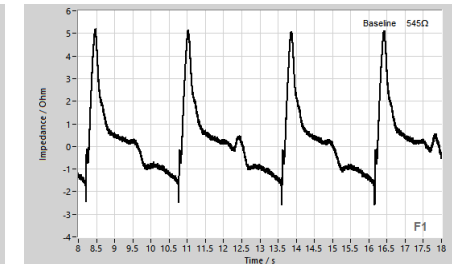
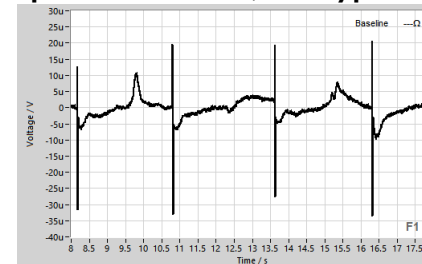
10μM d, l Sotalolol, cell type 1



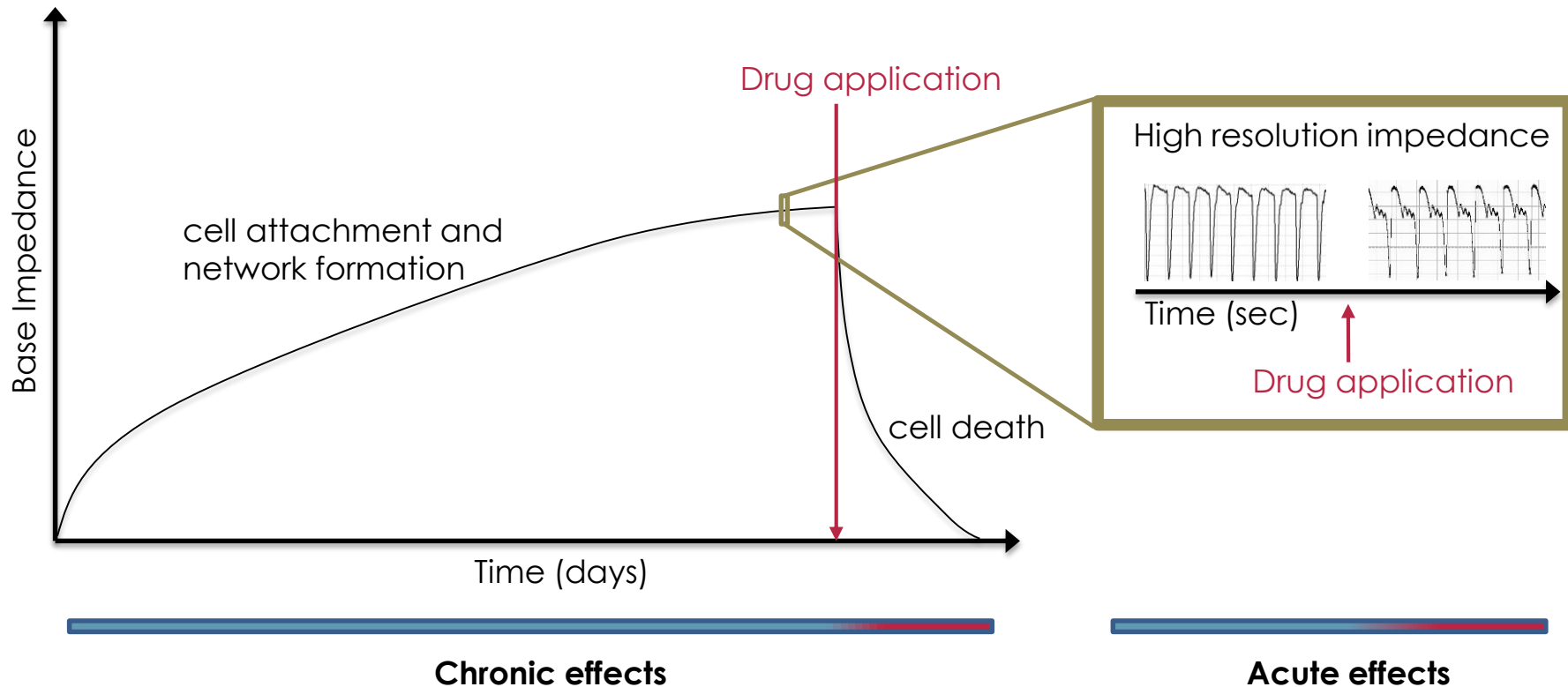
10μM Disopyramide, cell type 1



1μM Vandetanib, cell type 1

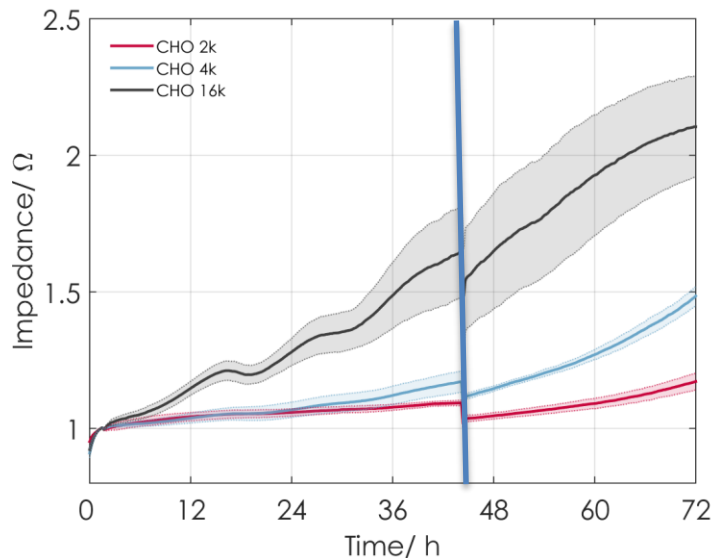


Long-term and short-term recording for chronic and acute effects



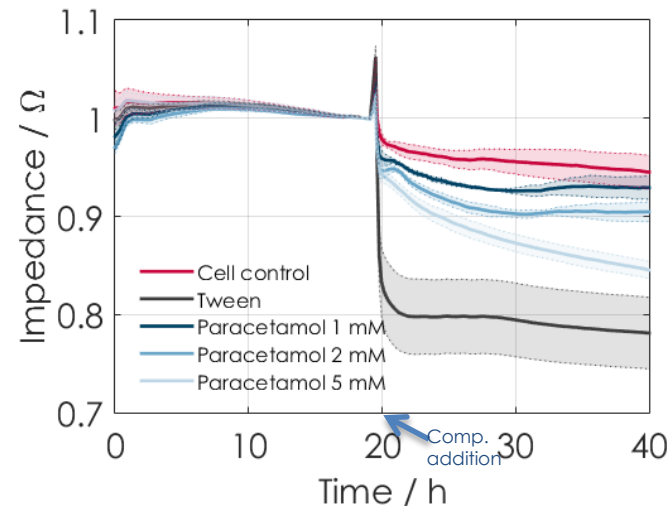
Cell adhesion and proliferation assays

Cell proliferation/ Cell QC



Cell proliferation of CHO cells, initially seeded cells per well were 2k, 4 k and 16k.
Blue line: medium exchange

Hepatotoxicity



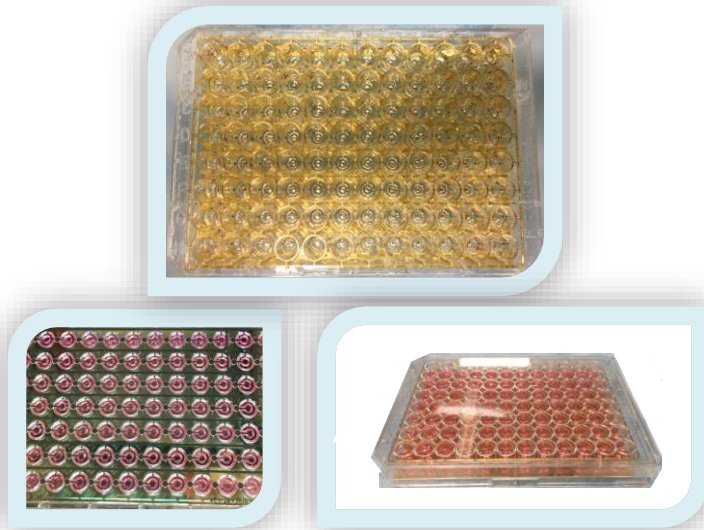
Patient specific hepatocyte-like cells treated with Tween as a control (complete cell death) and increasing Paracetamol concentrations. Note the concentration dependent hepatotox effect.



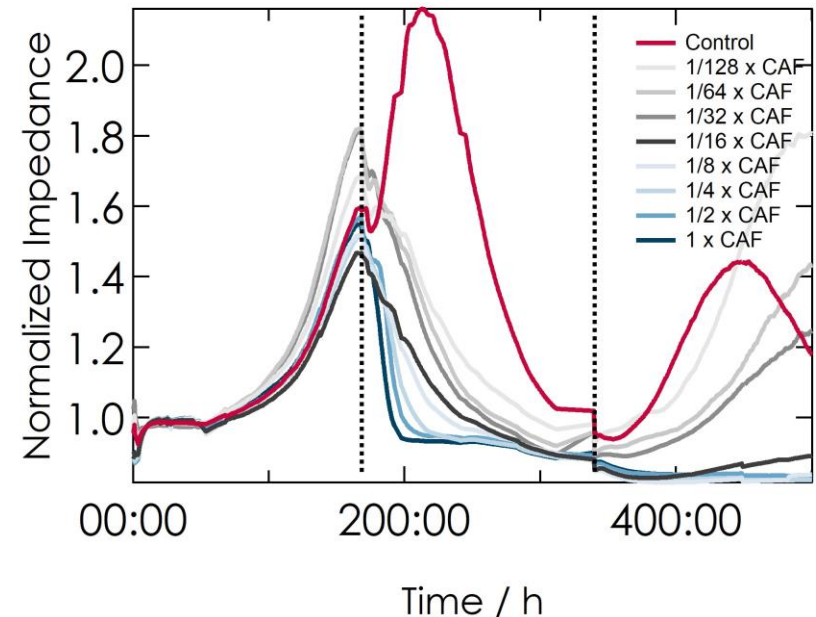
First technology to diagnose or exclude
Drug-induced liver injury (DILI)
in individual patients

Cell adhesion and proliferation assays

H8N8 for investigations of tumor cell recurrence



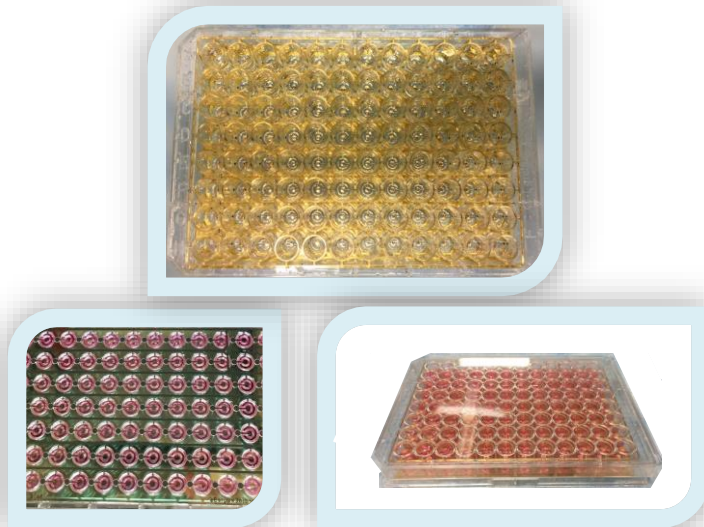
NSP®-96 transparent sensor plates allow imaging of seeded cells and long term recordings over weeks.



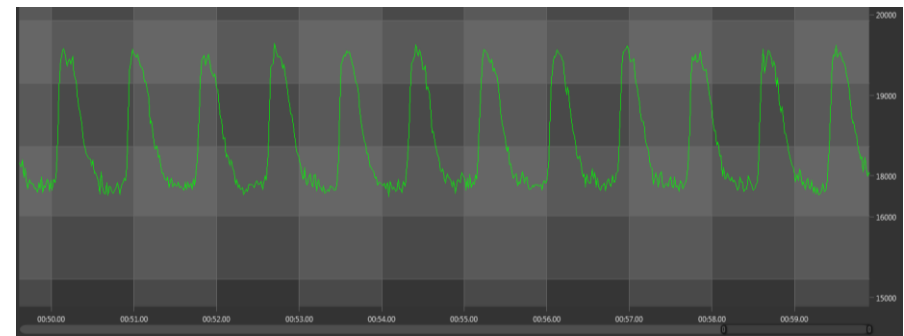
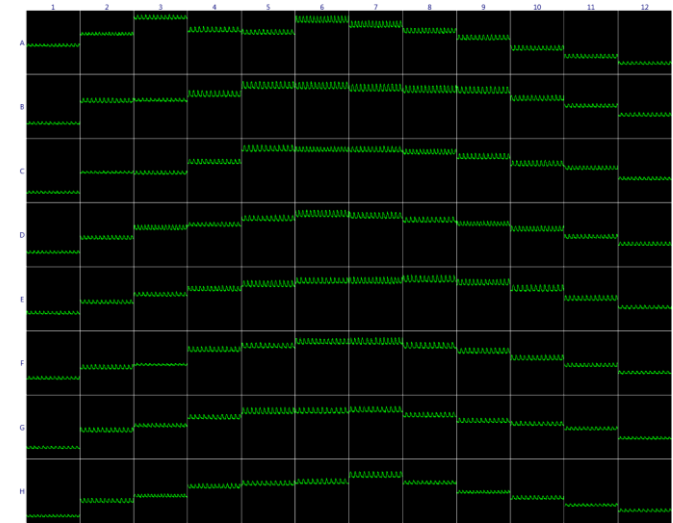
Long term monitoring of H8N8 tumor cells. 500 h monitoring of H8N8 cells, which have been developed as a tool to investigate tumor cell recurrence, chemoresistance and epithelial-to-mesenchymal transition (EMT). Cells were treated with increasing concentrations of CAF (cyclophosphamide, adriamycin (doxorubicin) and 5-fluoracil) on the CardioExcyte 96 during impedance measurements.

Transparent NSP-96 Sensor plates

Ca²⁺ imaging (FDSS Hamamatsu) on Cor.4U cells



NSP[®]-96 transparent sensor plates allow imaging of seeded cells and long term recordings over weeks.



Ca²⁺ imaging (FDSS Hamamatsu), Cor.4U
Kindly provided by Ralf Kettenhofen, Axiogenesis AG

Agenda

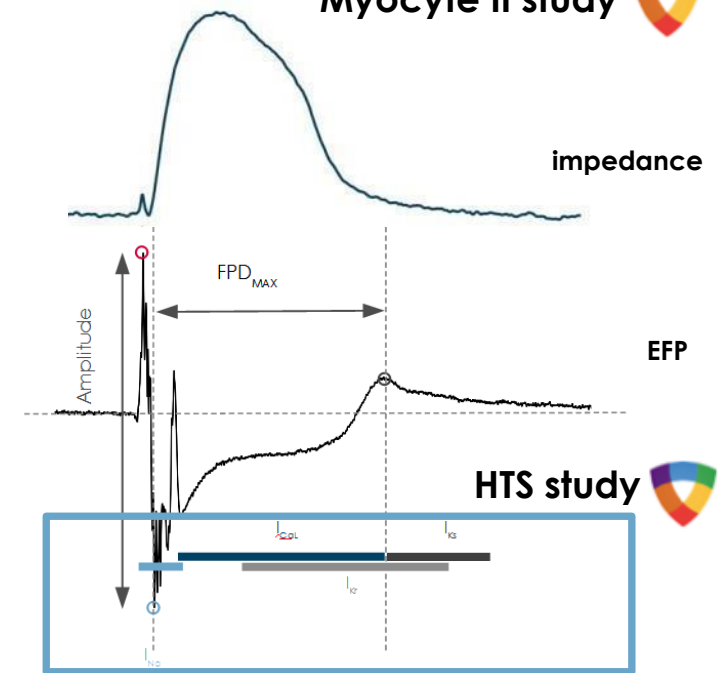
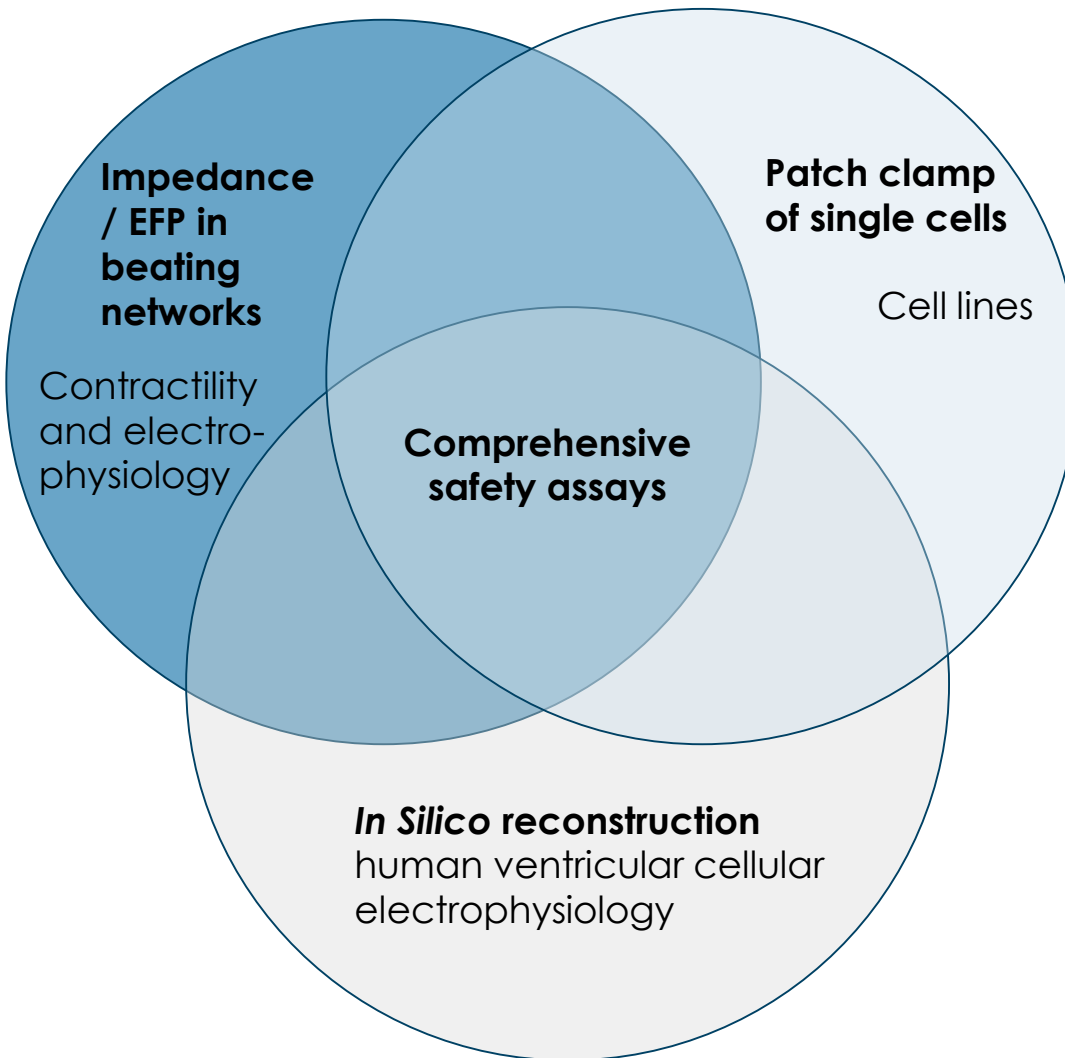
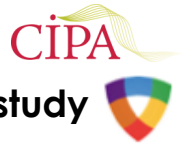
SyncroPatch 384 PE & cardiac
ion channels in cell lines and iPS CM

CardioExcyte 96 & cardiac
pharmacology in iPS CM

Summary

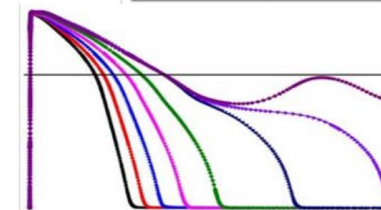


Holistic view on cardiac pharmacology

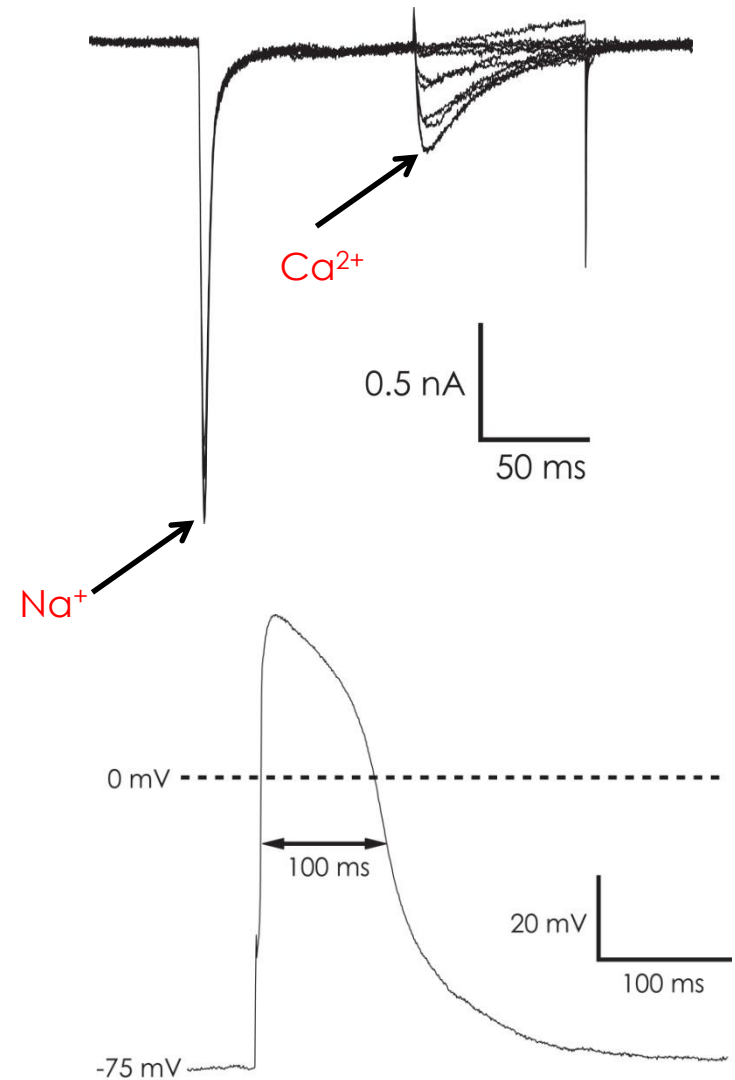
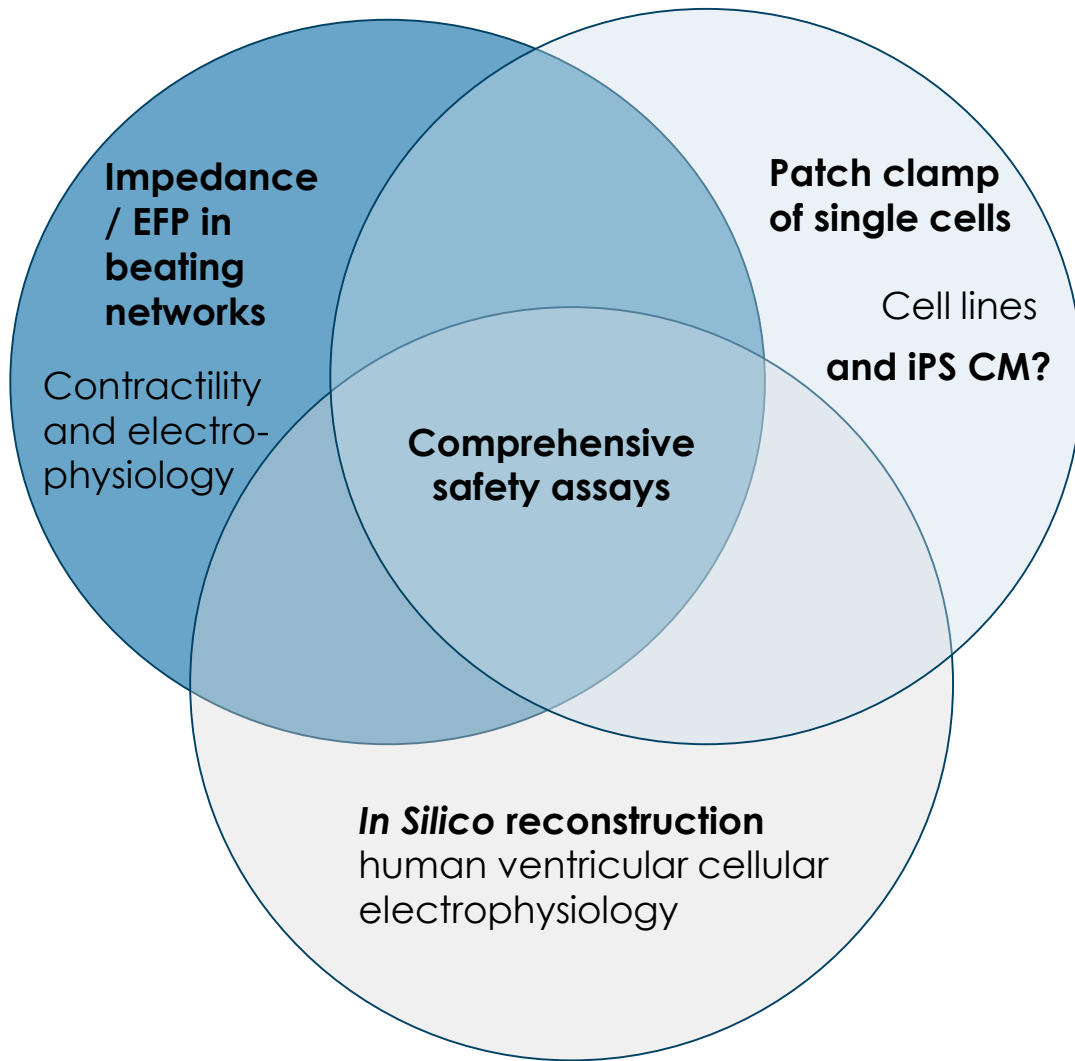


$$I_{stim} = C \frac{dV_m}{dt} + I_m$$

In silico
modelling
group



Holistic view on cardiac pharmacology



Summary

- ✓ We have established SOPs for APC and CardioExcyte 96 measurements in frame of the CiPA studies
- ✓ APC: Success rate for those targets were between 80 to 90% after applying the QC filters required from the CiPA guidelines
- ✓ Patchliner and SyncroPatch 384PE are equipped with features that are needed for investigating iPS CM:
 - ✓ Temperature control
 - ✓ Low cell consumption, GigaOhm seals
 - ✓ Current clamp
 - ✓ Dynamic clamp (ongoing project)

