

## CiPA HTS Study Progress at Nanion headquarters December 2016

### Nanion Technologies is an official test facility participating in the HTS study initiated by the CiPA consortium.

At Nanion headquarters in Munich, the scientists join the CiPA initiative with data of single cardiac ion channel patch clamp evaluations (HTS study on the SyncroPatch 384PE and Patchliner with stably transfected cell lines) as well as with data of impedance/ MEA recordings (CardioExcyte 96 Myocyte phase II study with human iPSC-derived cardiomyocytes).

Here, we present our preliminary data of the recent HTS CiPA study on the SyncroPatch 384PE & Patchliner.

### Evaluation of HTS Platforms for Assessing Drug Effects on Cardiac Currents: Nav1.5 Peak

All information from the CiPA guidelines were compressed in a channel specific (recording mode, buffers, etc.) operation procedure for each Nanion customer that participates in HTS CiPA study. The SOP was supplemented with experimental procedures

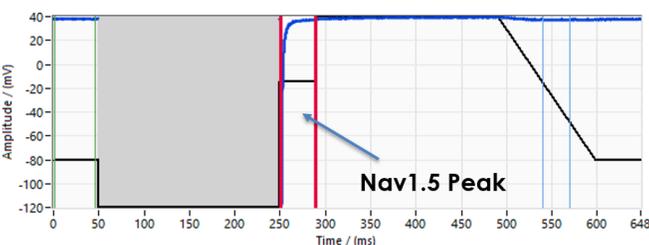


Fig. 1: Nav1.5 pulse protocol defined by CiPA

(trees) cell handling recommendations to guarantee identical conditions and less variability between labs.

In Fig. 1, the CiPA step-ramp Nav1.5 protocol is depicted. For analysis, the data were filtered according to different quality criterias (QC).

Fig. 2 shows corresponding Nav1.5 peak recordings on a SyncroPatch 384PE.

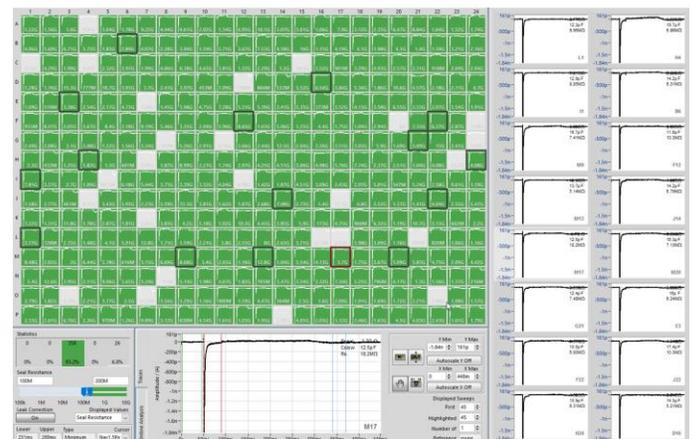
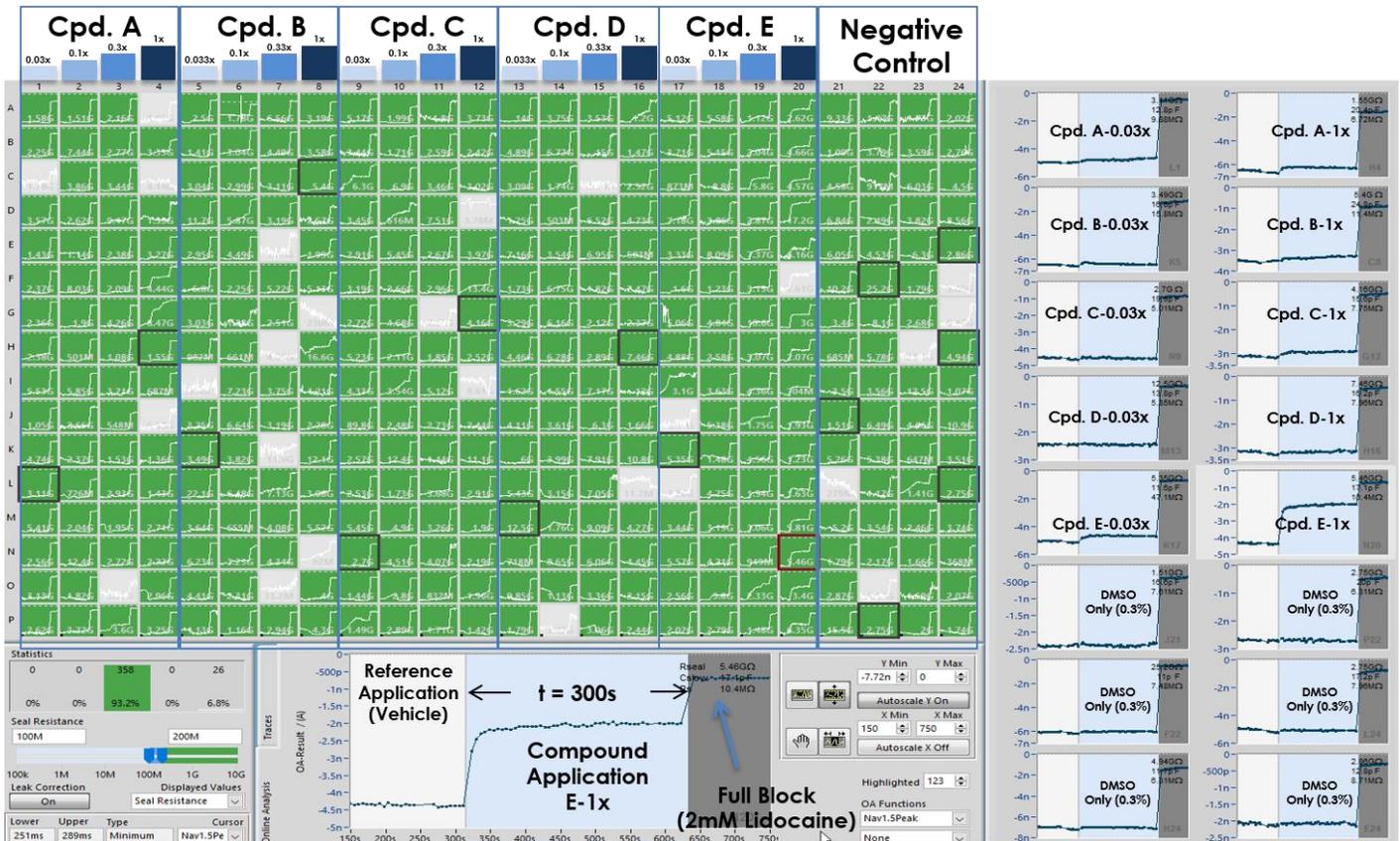


Fig. 2: Overview of one NPC-384 chip showing recordings of Nav1.5. in response to the CiPA voltage protocol following QC filters. Recording was performed on a single-hole chip on the SyncroPatch 384PE.

### Preliminary results on Nav1.5 peak current

Inward deflection represent activity of Nav1.5 expressed in a CHO cell line using the CiPA protocol.

Color coding reflects the seal resistance and reveals that 93.2 % of the cells pass the quality criteria required from the CiPA guidelines: A seal resistance > 200 MOhm, peak current > -200 pA, peak to leak



**Fig. 3:** Screenshot of an IT-Plot analysis on the SyncroPatch 384PE, applying CiPA compounds on Nav1.5

ratio > 25 %. Grey wells represent cells which did not pass the QC criteria.

We have executed four different runs to test all compounds. Success rates were above 91 % for all experiments.

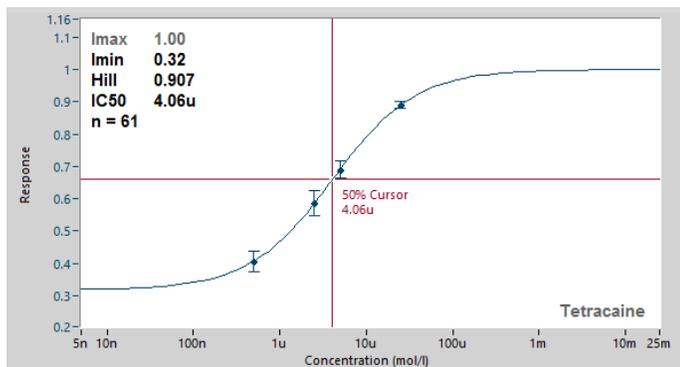
### CiPA compound recordings on Nav1.5 peak current

The plot of the Nav1.5 peak current over time (t) indicates the time course of applying five from eleven (Europe) CiPA compounds (Fig. 3). Recording was done on a single-hole chip.

For the whole chip, four different concentrations for each compound plus negative control were applied

on 384 cells at the same time. Each column represents one concentration with n=16 for each concentration.

The time course of application is highlighted on the bottom and on the right for 16 wells: After application of reference solution (containing 0.3 % DMSO) indicated by the white bar, the compound was applied for five minutes (light blue bar), after this a full block concentration of 2 mM Lidocaine (dark grey bar) was applied.



**Fig. 4:** Dose response curve of Tetracaine on Nav1.5

## Dose response curve of Tetracaine on Nav1.5 peak currents

Using the automated analysis software from Nanion, dose response curves were generated from CiPA compounds and positive controls. The negative controls show very little variation in terms of RunUp/Down and is below 5 % (not shown).

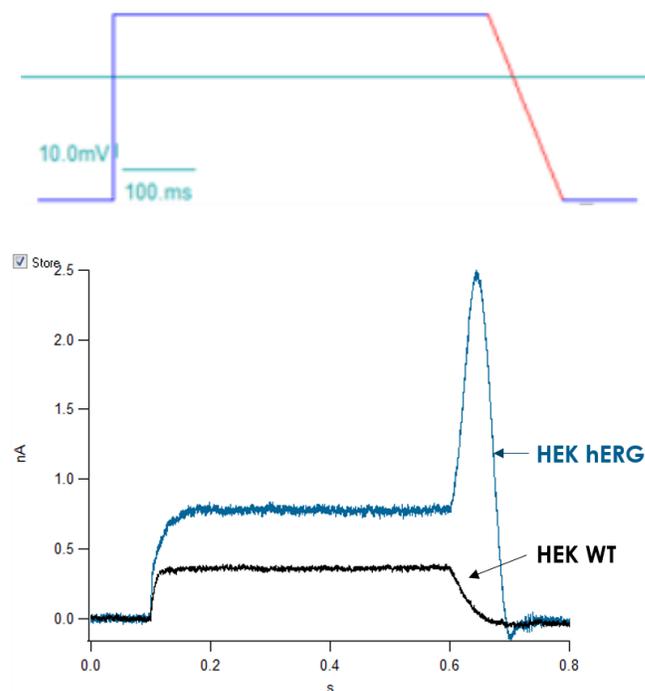
Tetracaine showed an IC<sub>50</sub> of 4.06 μM and a Hill coefficient of 0.907 using the CiPA protocol.

	SyncroPatch 384PE		Patchliner	
	RT	35 °C	RT	35 °C
hERG	✓		✓	✓
Kir2.1				
KvLQT1/KCNE1				
Kv4.3				
Nav1.5 (peak)	✓			
Nav1.5 (late)				
Cav1.2	✓			

**Table 1:** CiPA progress at Nanion headquarters December 2016. Compound delivery: November 2016.

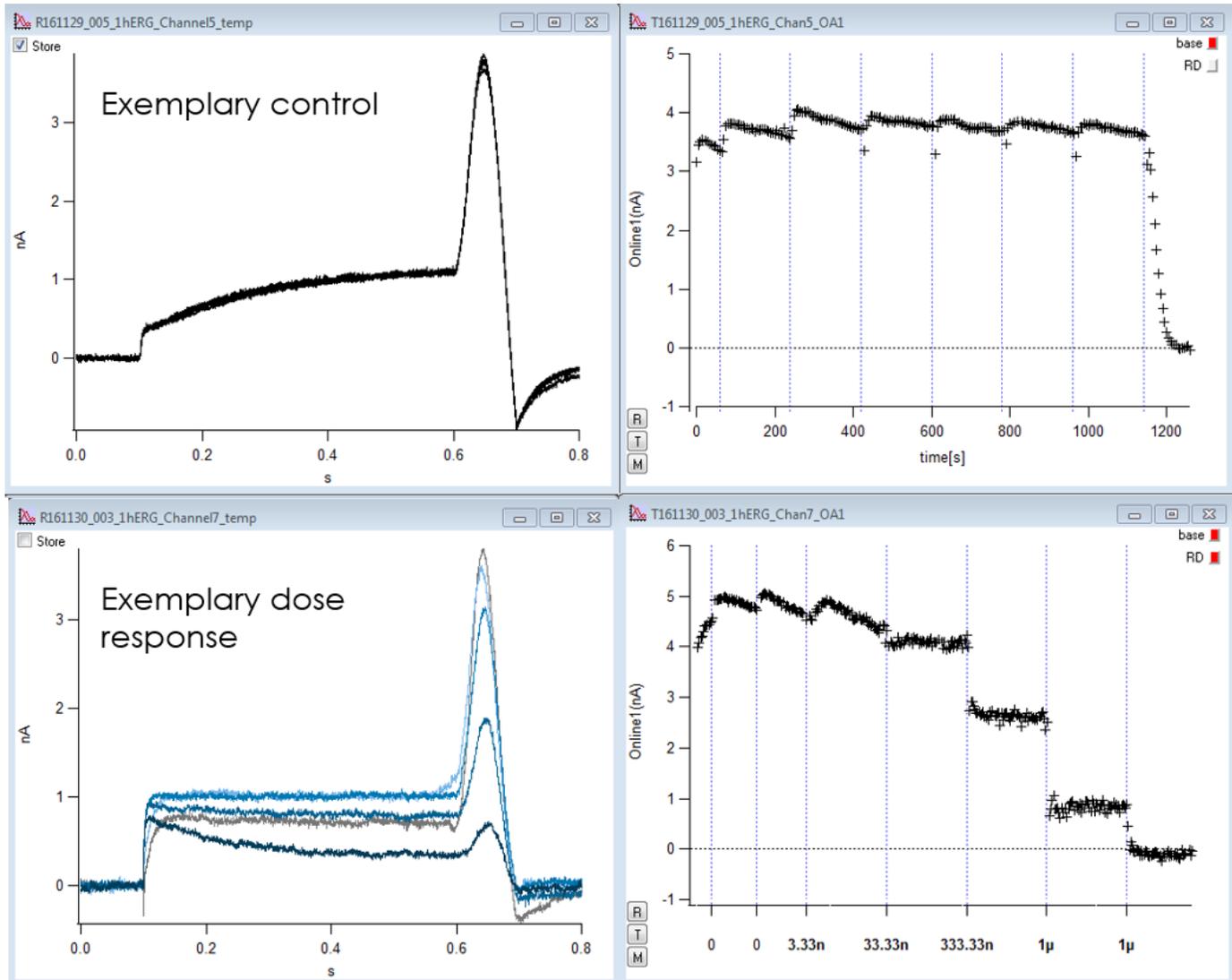
## hERG recordings on the Patchliner for CiPA

The SyncroPatch 384PE has been used to measure hERG, Nav1.5 peak current and Cav1.2. In addition, the Patchliner, which is a medium throughput device for recording up to eight cells simultaneously, has been used to measure hERG at room temperature and physiological temperature.



**Fig. 5:** Voltage protocol for hERG recordings on the Patchliner

For the hERG studies on the Patchliner, a stably-transfected HEK 293 cell line was used (Fig. 5). The compound application protocol set by CiPA was executed with four cumulative additions of CiPA compounds and a full block application with E4031. The CiPA step-ramp protocol results in stable hERG peak current recordings over 22 – 36 minutes.



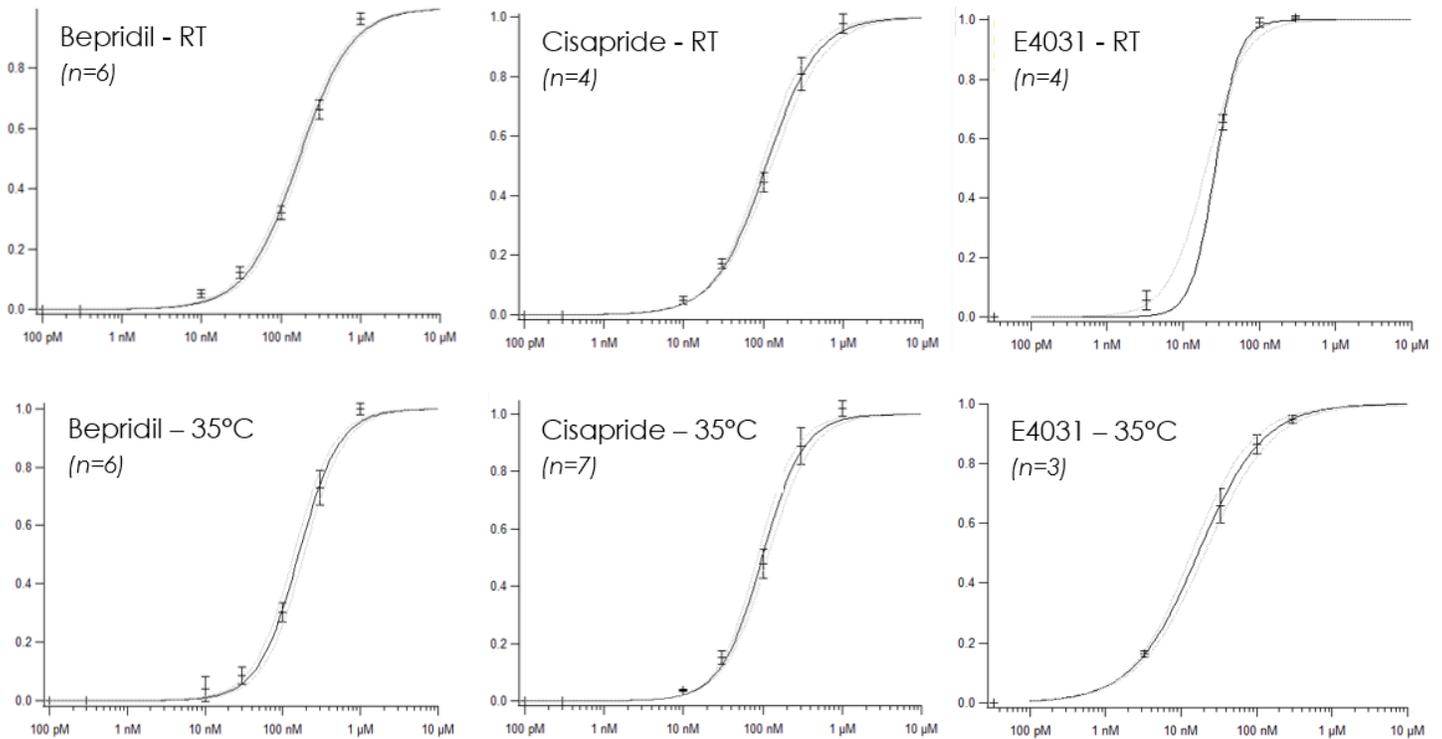
**Fig. 6:** Raw data and IT of Patchliner recording. CiPA compounds were applied on hERG expressing cells.



Sonja Stölzle-Feix, Co-chairing the HTS leadership team of CiPA & Director, Scientific Affairs at Nanion Technologies.

“Nanion’s APC platforms allow for high quality patch clamp recordings and are proven to allow a straightforward implementation of CiPA protocols in labs worldwide.

Nanion customers are being supported by our electrophysiology team with ready-to-go CiPA conform and platform specific protocols, trees and experimental setups. We see that the success of this highly significant international project relies on the collaboration between the CiPA participants and the HESI/CiPA consortium and we are happy to assist in any way we can.”



**Fig. 7:** Reference compounds for hERG were successfully tested at RT and physiological temperature.

	Bepridil	Cisapride	E4031
IC <sub>50</sub> at RT	168 nM	109 nM	26 nM
IC <sub>50</sub> at 35 °C	161 nM	98 nM	17 nM

**Table 2:** IC<sub>50</sub> values of hERG reference compounds measured on the Patchliner at RT and 35 °C.

## The Patchliner

The Patchliner® is a fully automated patch clamp platform offering medium throughput and vast experimental freedom.



## The SyncroPatch 384PE

- Automated HTS Patch Clamp
- Modular approach
- 384 recordings in parallel,
- 384 high-grade patch clamp amplifiers
- Supports giga-seal recordings

- Uses borosilicate recording substrates
- Routinely > 85 % success rate
- Voltage-clamp maintained at all times
- Optional Current-Clamp mode
- Optional Temperature Control

