

Reliable Identification of hERG Liability in Drug Discovery by Automated Patch Clamp

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Introduction

The Comprehensive in Vitro Proarrhythmia Assay (CiPA) was initiated in 2013 to improve pre-clinical drug safety paradigms. The aims of the initiative are not only to ensure that drugs which reach the market are safe, but also to reduce the number of false positives to ensure that more potentially useful (and safe) drugs reach the market. As part of this initiative, compounds representing high, intermediate, and low pro-arrhythmic risk categories were tested across multiple sites and automated patch clamp (APC) platforms. Resulting IC₅₀ values varied between platforms, which could be due to non-specific binding of compounds in well-based platforms since they don't maintain a constant flow of compound-containing solution to replenish losses to fluidics and well walls.

Here, we investigated non-specific binding using hydrophobic ("sticky") compounds to assess the reliability of our experimental routine by analyzing IC₅₀ values of CiPA compounds. We used a state-of-the-art liquid handling robot encompassing a 384-channel patch clamp module for compound preparation and high throughput APC recordings. Glass-coated compound plates were used to prevent absorption of hydrophobic compounds. After compound preparation, the user-unattended experiments were executed and IC₅₀ values of the compounds were measured and compared to literature values.

High throughput screening on the SyncroPatch 384i



Nanion's patch clamp module (left) for the SyncroPatch 384i, enabling 384 parallel recordings. The robotic system is based on the Biomek i5 state-of-the-art liquid handler (right).



hERG recordings using different voltage protocols

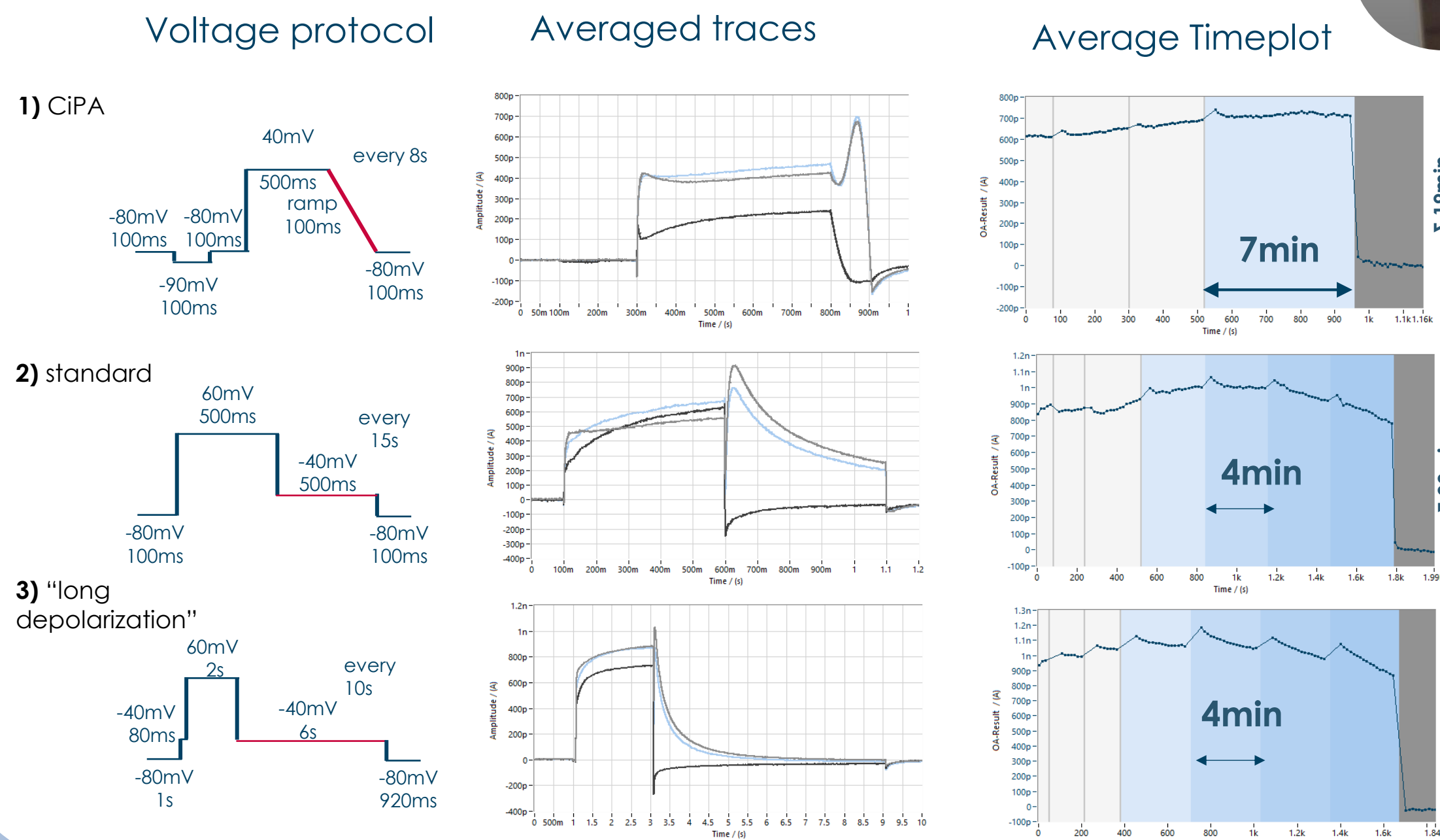


Fig. 1 Diverse hERG voltage protocols, example traces and corresponding plots of current amplitude vs. time in control (grey), vehicle control (4 times, cumulative for protocols 2, and 3; single addition in 1; shades of blue) and full block (dark grey).

hERG – reliable assay due to stability of recordings

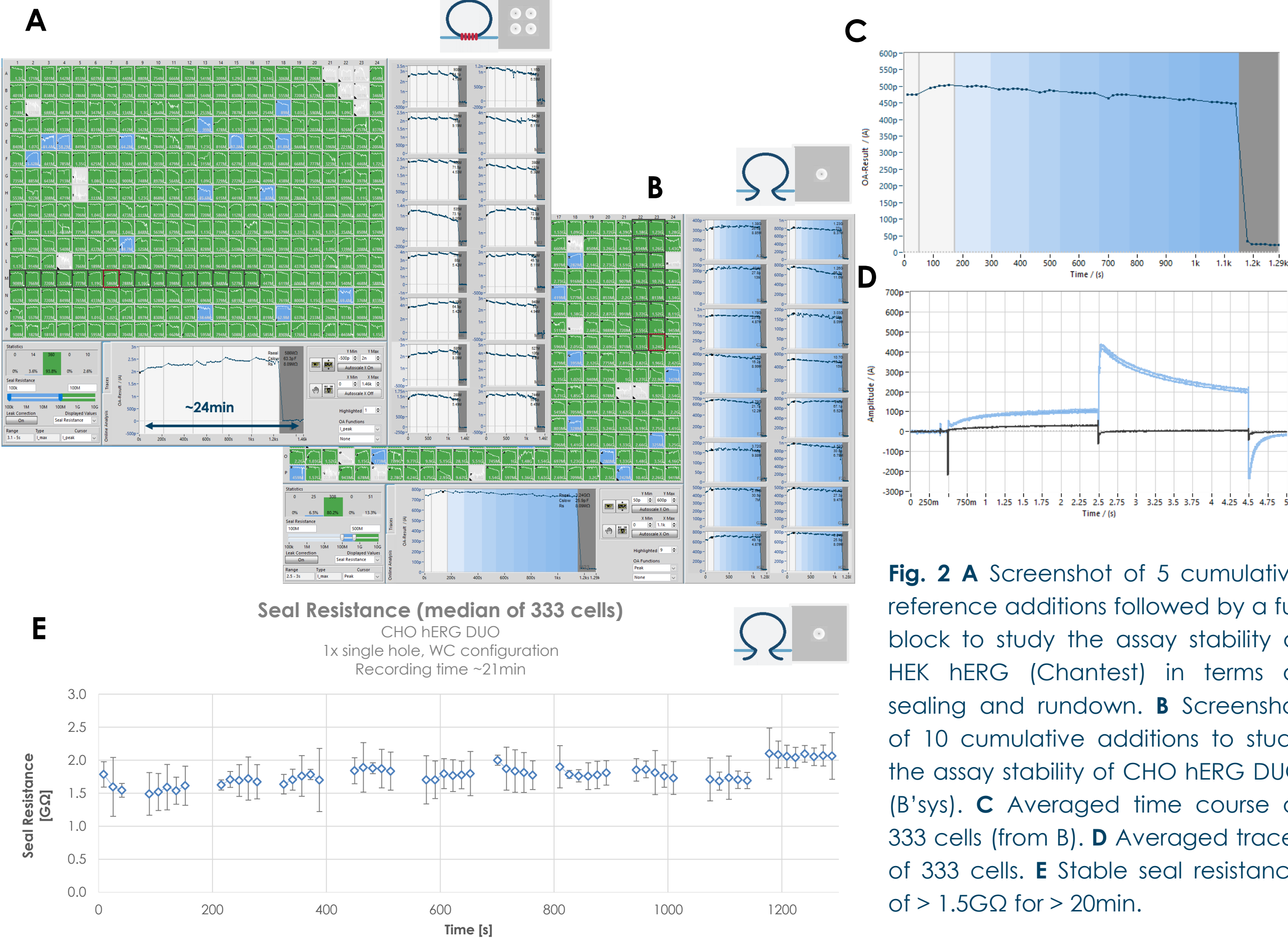
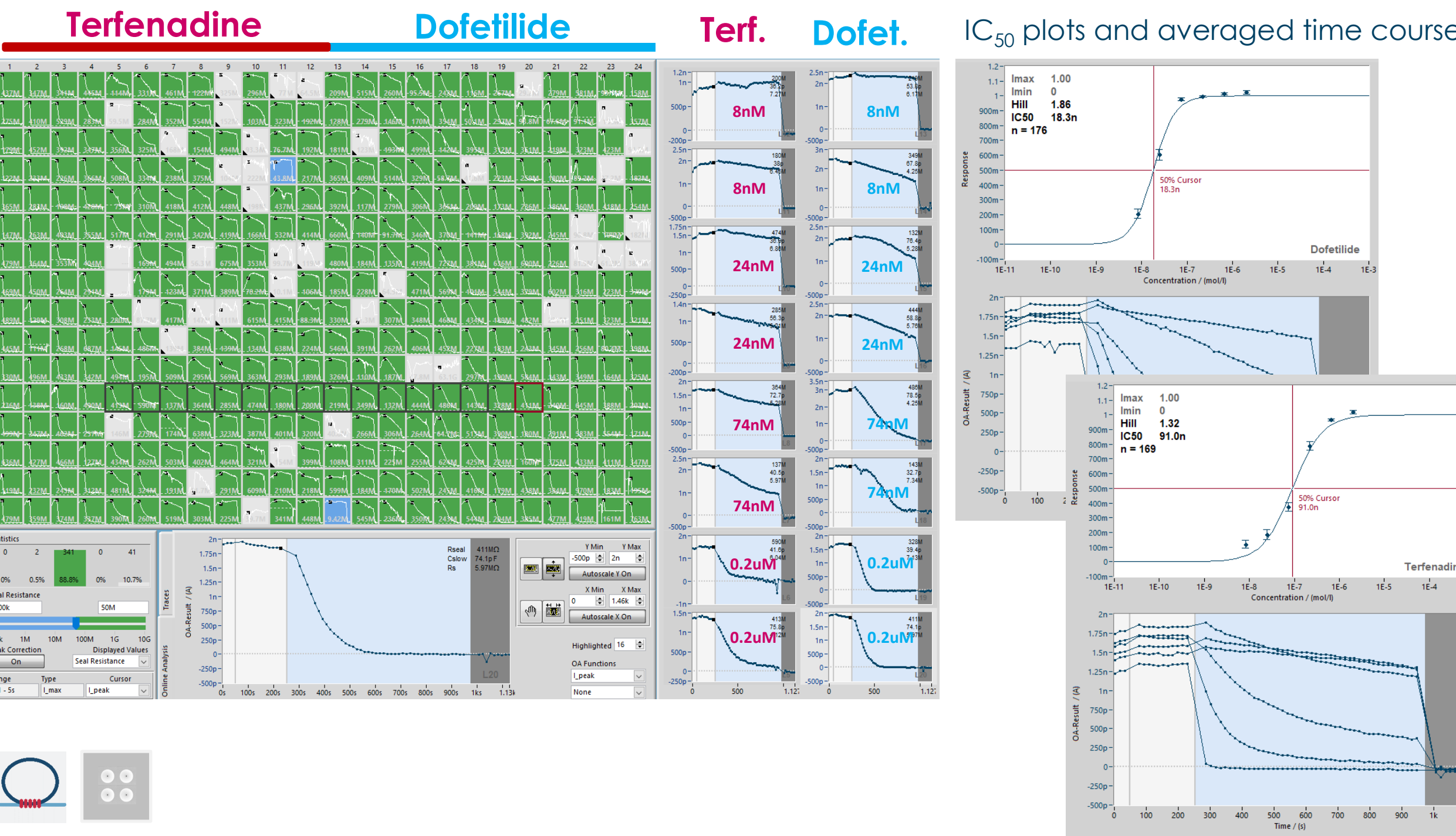


Fig. 2 **A** Screenshot of 5 cumulative reference additions followed by a full block to study the assay stability of HEK hERG (Chantest) in terms of sealing and rundown. **B** Screenshot of 10 cumulative additions to study the assay stability of CHO hERG DUO (B'sys). **C** Averaged time course of 333 cells (from B). **D** Averaged traces of 333 cells. **E** Stable seal resistance of > 1.5GΩ for > 20min.

Slow inhibitors need an extended incubation time



Cpd	IC ₅₀ Literature* (CiPA)	IC ₅₀	Wells included	Data points included	Success Rate [%]
Dofetilide	0.036	0.018	176	176	92
Terfenadine	0.052	0.092	169	169	88

Fig. 3 Terfenadine and Dofetilide were tested as 6pt-IC₅₀ across the plate on HEK hERG using a multi hole 4x chip and perforated patch mode. To reach a steady state at lower concentrations an extended compound incubation time was needed (>12min). If this is not done, the potency of these compounds is underestimated.

SyncroPatch 384i liquid handling of sticky compounds and the effect of labware material on calculated IC₅₀ values



The SyncroPatch 384i platform allows for unattended runs in a fully automated manner. Independent movements of gripper and pipette head and a selective tip option for serial compound dilution enable a workflow that fits an HTS screening environment.

Table 1: The non-specific binding of compound to labware was investigated. IC₅₀ values of astemizole were evaluated after storing the compound between 5 min and 3 h in teflon reservoirs, Eppendorf cups and glass vials. IC₅₀ values were recorded on the Patchliner and the SyncroPatch 384PE.

Cpd	IC ₅₀ [uM] Literature* CiPA	IC ₅₀ [uM] SyncroPatch	Success Rate
Astemizole	0.004	0.037	78%
Bepidil	0.159	0.242	78%
Cisapride	0.024	0.029	94%
Dofetilide	0.036	0.021	94%
E4031		0.020	78%
Flecainide	1.49	0.920	100%
Quinidine	0.716	0.761	94%
Ranolazine	10.7	5.19	91%
Terfenadine	0.052	0.020	69%
Verapamil	0.250	0.350	78%

Fig. 4 Success rates and IC₅₀ values compared with literature values published by Chantest within the CiPA Initiative*. Data was recorded using the CiPA Protocol (1) shown above in Fig. 1.

Instrument	Labware	Time between cpd prep and application	IC50	n
384PE	Teflon	~5min	30nM	180
384PE	Teflon	~30min	83nM	177
384PE	Teflon	~2h	151nM	168
Patchliner	Eppi	~5min	37nM	5
Patchliner	Eppi	~2h	63nM	4
Patchliner	Eppi	~3h	209nM	3
Patchliner	glass vial	~5min	35nM	6
Patchliner	glass vial	~2h	37nM	2
Patchliner	glass vial	~3h	37nM	4

Conclusions

- The 384-well APC system used in our study (SyncroPatch 384i) is well suited to perform safety pharmacological studies within the guidelines of the CiPA initiative.
- Different voltage protocols can be used to record hERG, experiments are stable regardless of protocol used.
- Variability of IC₅₀ values of drugs in different platforms within the CiPA study could have been caused by non-specific binding of compounds
- Caution should be taken on the handling and storage conditions of the compounds, as well as exposure times of the compounds during the measurement.
- When using teflon or plastic labware, experiments should be carried out immediately after compound preparation.

*Ref for literature values: <http://www.onemillionsolutionsinhealth.org/wp-content/uploads/2014/11/ChanTest-Data-Analysis-Summary.pdf>