# Reliable identification of cardiac liability in drug discovery using automated patch clamp: Experimental and technical considerations for high throughput recordings of $Na_V1.5$ and hERG

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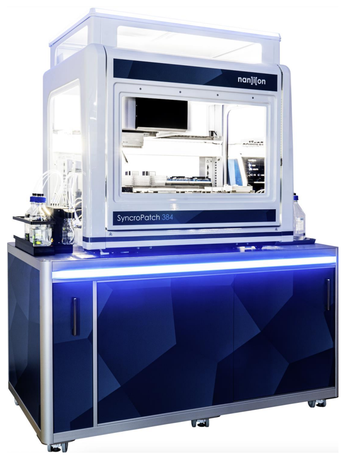
## Introduction

The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) aim is to improve specificity compared to *in vitro* hERG and *in vivo* QT studies. Automated patch clamp (APC) instruments are increasingly adopted for cardiac safety measurements on hERG, $Na_V1.5$ and $Ca_V1.2$, thus requiring standardized experimental protocols and technical specifications, e.g. temperature control, accessibility of recording solutions to allow for sample collection or Liquid Junction Potential (LJP) correction for accurate voltage control.
Here, we identified parameters influencing $IC_{50}$ determination of compounds on hERG and $Na_V1.5$ currents recorded using APC. For example, we found that although voltage protocol did not affect the $IC_{50}$ of hERG compounds, both voltage protocol and holding potential could affect $IC_{50}$ on $Na_V1.5$ peak currents.




**SyncroPatch 384**
384 recording channels

Temperature could also influence $IC_{50}$ values of compounds tested on $Na_V1.5$ peak. It is critical to maintain a constant recording temperature of the system either by cooling where room temperature fluctuates, or heating to record at physiological temperature. Experimental parameters such as incubation time also influenced $IC_{50}$ values for both hERG and $Na_V1.5$ peak currents and whereas compounds such as bepridil reached steady state within approximately 5-6 mins, sticky compounds such as terfenadine required longer incubation times. In keeping with this, the open-well design of the patch clamp chip makes it possible to collect samples directly from the measurement site. This allows the direct measurement of actual compound concentration at the cell.


**Patchliner + Dynamite[8]**
8 recording channels + Dynamic clamp

## Effect of temperature on biophysical properties and $IC_{50}$

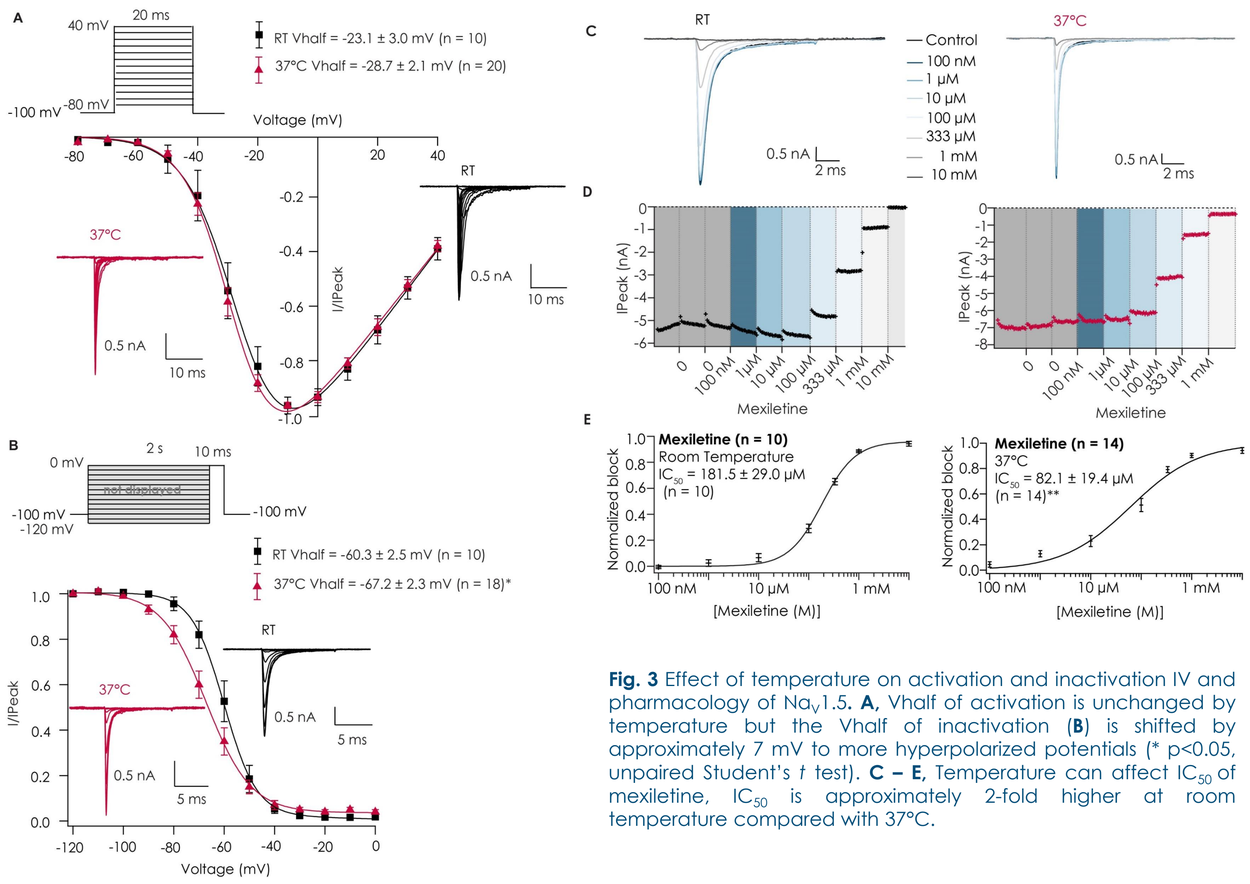



**Fig. 3** Effect of temperature on activation and inactivation IV and pharmacology of $Na_V1.5$. **A**, Vhalf of activation is unchanged by temperature but the Vhalf of inactivation (**B**) is shifted by approximately 7 mV to more hyperpolarized potentials (* $p<0.05$, unpaired Student's *t* test). **C – E,** Temperature can affect $IC_{50}$ of mexiletine, $IC_{50}$ is approximately 2-fold higher at room temperature compared with 37°C.

## Effect of voltage protocol on $Na_V1.5$

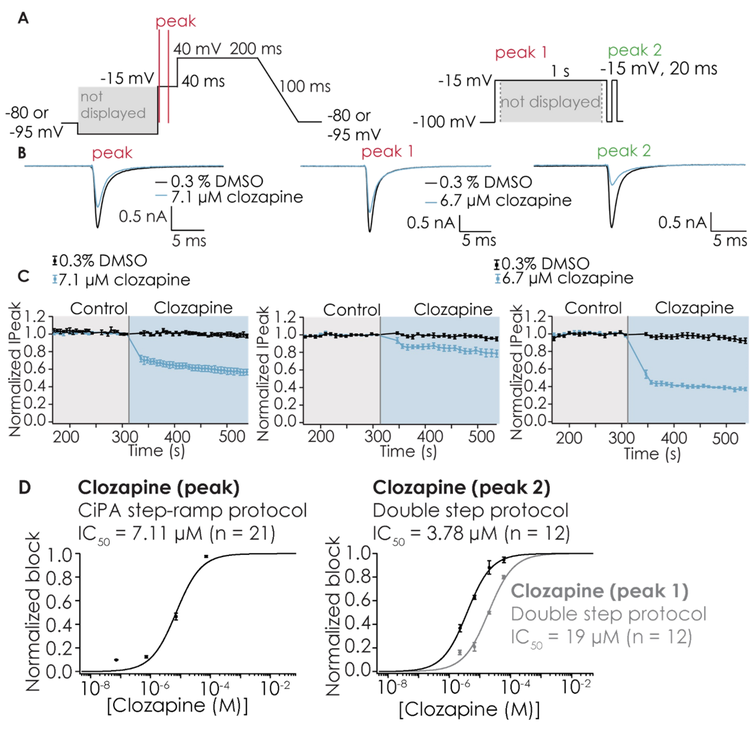



**Fig. 1 A**, CiPA step-ramp (left) and double step (right) voltage protocols. $Na_V1.5$ is reliably recorded (**B**,**C**), there was no rundown of peak current in control (vehicle) recordings (**C**). Voltage protocol can have an effect on $IC_{50}$, in particular when compounds display state dependence (**C**,**D**).

**Recommended reading:**
https://doi.org/10.1016/j.vascn.2021.107125

https://doi.org/10.1016/j.vascn.2020.106884

## Incubation time and reproducibility

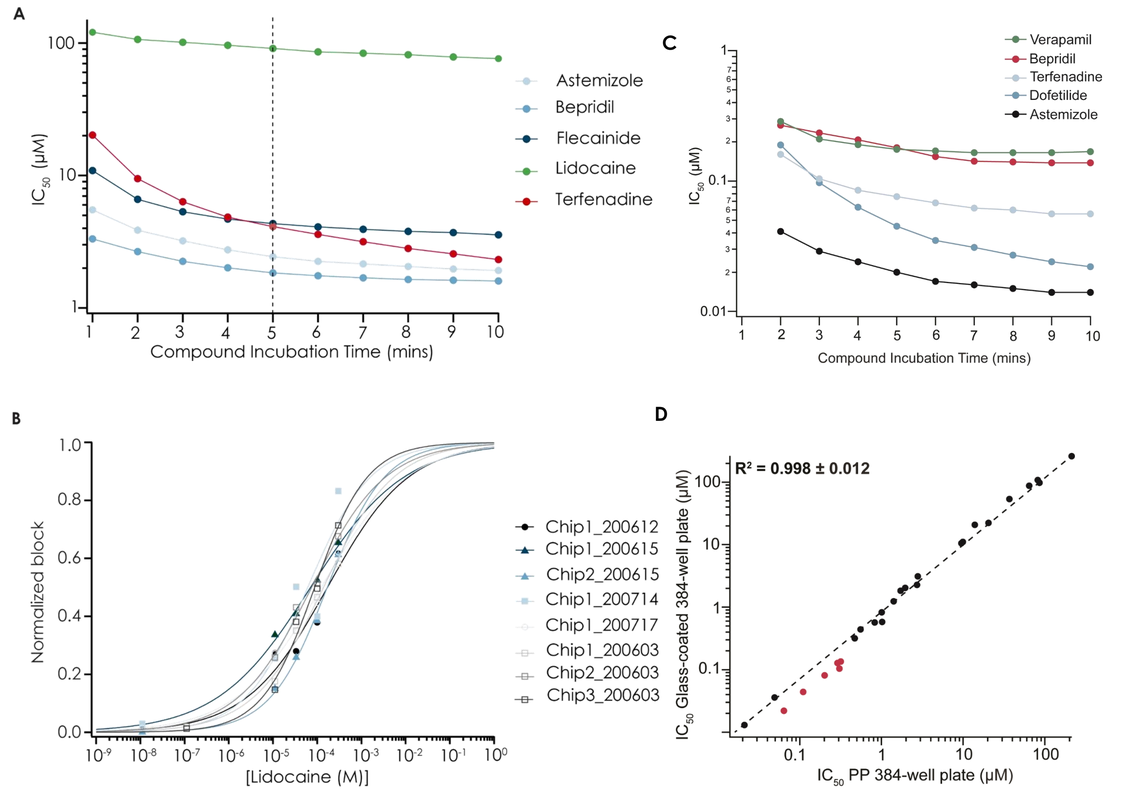



**Fig. 4 A,** Effect of incubation time on calculated $IC_{50}$ values on $Na_V1.5$ for 5 compounds. For most compounds, 5 min incubation in compound was sufficient to reach steady state but for sticky compounds, such as terfenadine, 9 or 10 min incubation was required. **B**, Data was reproducible: concentration response curves for lidocaine from different chips and different days overlaid almost exactly. **C** Compound incubation time plotted against $IC_{50}$ values for hERG. 4-point $IC_{50}$ values were calculated. Single concentrations were added to each well, compound applications were performed twice with the 2nd addition after 5 min. All compounds except for dofetilide reached steady state within 7 min, dofetilide required 10 min. **D** Correlation of $IC_{50}$ values gained with 27 compounds that were stored in glass coated 384-well plates or PP 384-well plates correlated well, the $R^2$ was calculated to be 0.998 ± 0.012. Biggest discrepancies of 2.3–2.9 fold were seen with astemizole, cisapride, domperidone, droperidol, pimozide and terfenadine (shown as red markers).

## Effect of voltage protocol on hERG

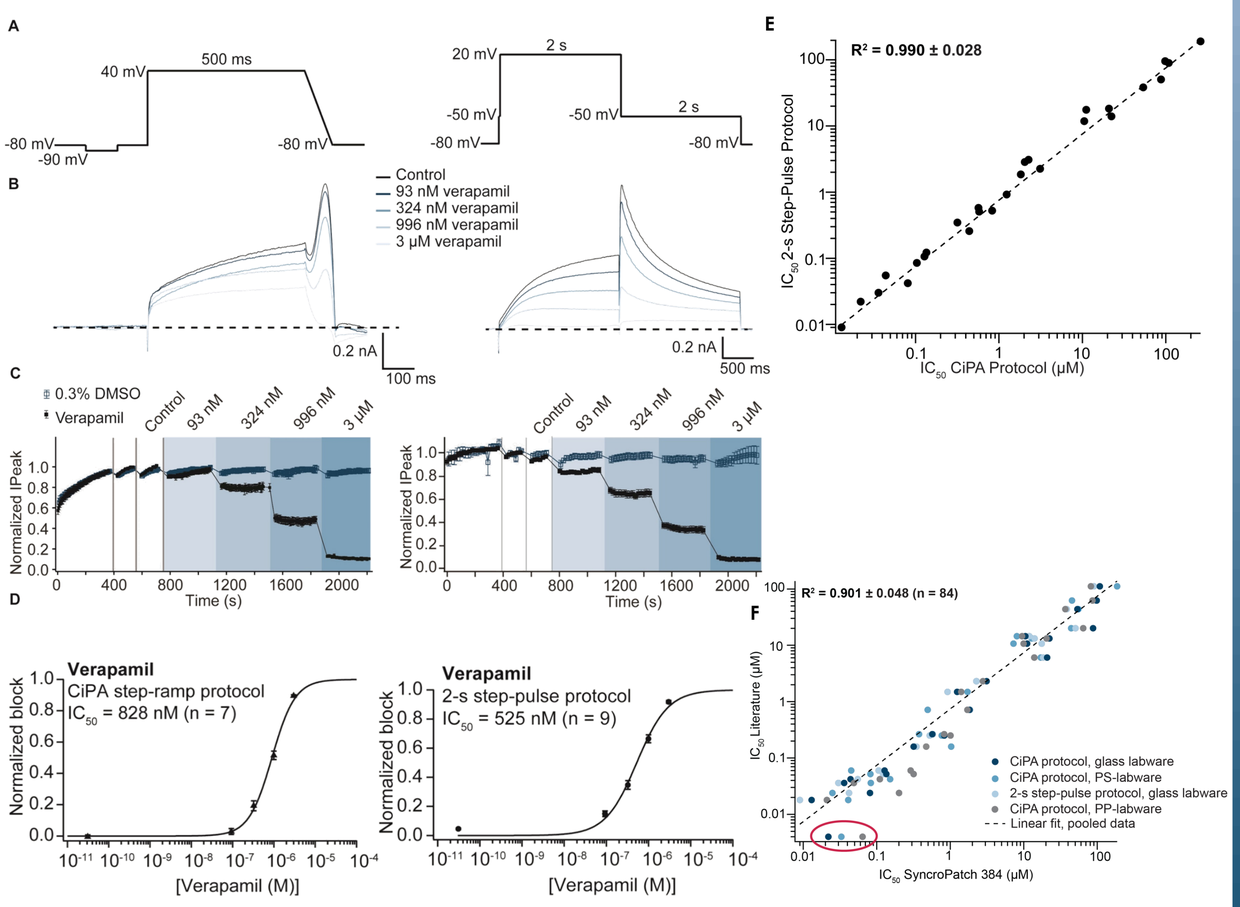



**Fig. 2. A,** hERG voltage protocols "CiPA" step-ramp (left) and "2-s step-pulse" (right) and corresponding mean data traces **(B). C,** Normalized peak current in control (grey), vehicle control and increasing verapamil concentrations (shades of blue). DMSO control (open blue squares) is also shown for comparison for n = 6 wells for the CiPA step-ramp and n = 9 wells for the 2-s step-pulse protocol. **D,** Estimated $IC_{50}$ values gained from the CiPA protocol were 828 nM (n =7) and 525 nM (n = 9) when using the 2-s step-pulse protocol. **E,** Correlation of $IC_{50}$ values calculated from block potencies of 27 compounds as calculated from peak current responses elicited either by the CiPA voltage protocol or the 2-s step-pulse protocol. The $R^2$ was calculated to be 0.990 ± 0.028 showing excellent correlation. Experiments were performed as a cumulative 4-point concentration application with a compound equilibration time of 5 min, CHO hERG Duo cells were used. **F,** Correlation of literature values[1] and HTS data from 3 sites. Pearson's correlation coefficient was $R^2$ = 0.901 ± 0.048 showing excellent correlation. The points circled in red are astemizole. [1]Kramer J., et al., 2013 doi: 10.1038/srep02100

## Sample collection - The prerequisite for concentration verification

From: *ICH E14/S7B Implementation Working Group:*
*Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential - Questions and Answers - Draft Version 2020*

>> *Both nominal and measured concentrations should be reported.*

- *Validated analytical method should be applied to solution collected from test chamber*
- *Measured concentrations should be used for $IC_{50}$ determinations if values differ significantly*

**Fig. 5 Sample collection on SyncroPatch 384** allows concentration verification as outlined by the ICH E14/S7B Implementation Working Group.

## Conclusions

- The $Na_V1.5$ CiPA step-ramp protocol is ideal for safety pharmacology testing allowing effects on both peak and late current to be studied. Voltage protocol does not affect $IC_{50}$ values measured on hERG.
- Temperature affects biophysical properties and pharmacology and should be carefully controlled. Where possible, experiments should be performed at physiological temperature.
- For $Na_V$ 1.5 measurements, a minimum compound incubation time of 5 min is required for reliable $IC_{50}$ measurement, longer is preferred for sticky compounds. For hERG, we recommend > 6 min.
- The use of standardized protocols for APC experiments e.g. voltage protocol, incubation time, replicate number etc, is critical to be able to compare $IC_{50}$ values across platforms, sites and with literature values.