# Application Note

Channel: $Na_V1.5$
Cells: CHO & HEK
Tools: SyncroPatch 384i

# $Na_V1.5$-ΔKPQ late $I_{Na}$ current properties and pharmacology on the SyncroPatch 384i

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## Summary

The cardiac late Na current (late $I_{Na}$) generates persistent currents throughout the plateau phase of the cardiac action potential. Several mutations in the SCN5A gene cause a form of hereditary long QT syndrome (LQT3)[1-3]. The ΔKPQ mutation deletes residues Lys 1505, Pro 1506 and Gln 1507, resulting in a sustained, non-inactivating current during long (over 50 ms) depolarizations[1,2]. This sustained current causes prolongation of the action potential which can result in fatal ventricular arrhythmias such as Torsade de Pointes (TdP)[1].

One aim of the Comprehensive *In Vitro* Pro-arrhythmia Assay (CiPA) initiative is to improve drug safety testing in pre-clinical development by evaluating the pro-arrhythmic risk of a compound[4,5]. Validation studies confirm that testing the effect of compounds on an increased number of human cardiac ion channel currents including $I_{Na}$ ($Na_V1.5$ peak and late current) as well as $I_{Kr}$ (hERG) leads to improved prediction of their clinical risk. Late $I_{Na}$ can be recorded in WT $Na_V1.5$ channels using the toxin ATX-II or veratridine, or using a cell line with LQT3 mutations in $Na_V1.5$ without the need for pharmacological enhancement. The latter might also reduce the risk of cross-reactions between late-current enhancers and test compounds.

Here we present data collected on the SyncroPatch 384i showing the peak and late $I_{Na}$ current recorded from WT and $Na_V1.5$-ΔKPQ cell lines. Peak current could be reliably recorded from both cell types. In WT cells, late $I_{Na}$ was negligible in the absence of ATX-II, whereas the late $I_{Na}$ from $Na_V1.5$-ΔKPQ cells could be reliably recorded. Peak current from WT, and peak and late $I_{Na}$ from $Na_V1.5$-ΔKPQ was inhibited by ranolazine and mexiletine and $IC_{50}$ values agreed well with the literature[6].

## Results

HEK cells expressing $Na_V1.5$-ΔKPQ and CHO cells expressing $Na_V1.5$-WT were recorded on the SyncroPatch 384i with good success rates. Peak currents with an amplitude of -1.6 ± 0.1 nA (n = 219) were recorded from WT cells and -5.0 ± 0.1 nA (n = 310) from $Na_V1.5$-ΔKPQ cells using single hole plates. The late $I_{Na}$ current was negligible in WT cells using the step-ramp voltage protocol shown in Figure 1 (-35 ± 1.8 pA, n = 58) but was reliably recorded using the same protocol from the $Na_V1.5$-ΔKPQ cells (-176 ± 63 pA, n = 74; QC parameters: $R_{seal}$ >400 MΩ, late $I_{Na}$ >-100 pA) using single hole chips. The late $I_{Na}$ current was increased and, therefore, success rate improved using multi-hole NPC-384 chips with 4 holes per well (4x). Using this type of chip, late $I_{Na}$ current amplitude was increased to -405 ± 14 pA, n = 167; Figure 1; QC parameters: $R_{seal}$ >250 MΩ, late $I_{Na}$ >-200 pA).

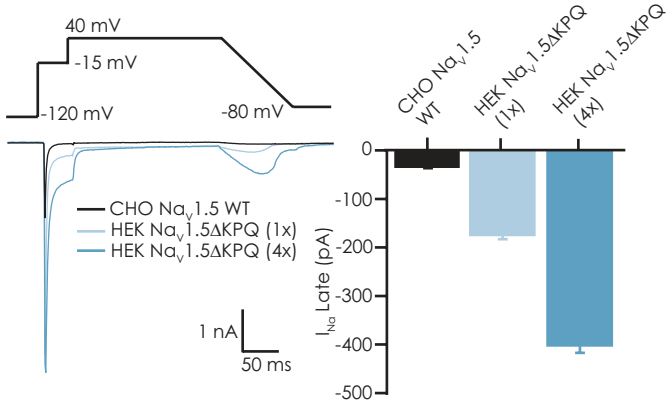


**Figure 1: Current recorded from WT and $Na_V1.5$-ΔKPQ expressing cells. A** Raw current traces from CHO $Na_V1.5$-WT (black), and $Na_V1.5$-ΔKPQ (blue) using either single hole (1x) or multi-hole (4x) chips. **B** Bar graph showing current amplitude of late $I_{Na}$ from WT and $Na_V1.5$-ΔKPQ. Late $I_{Na}$ was negligible in WT cells but could be reliably recorded in $Na_V1.5$-ΔKPQ.

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As the ΔKPQ mutation affects the voltage-dependence of $Na_V1.5$ channels it was important to determine the biophysics of this cell line on the SyncroPatch 384i platform. Activation and inactivation of peak currents of $Na_V1.5$-ΔKPQ were recorded using voltage-step protocols. The activation plot is shown in Figure 2 and fit with a Boltzmann equation. The $V_{0.5}$ of activation of the mean current IV was -34.5 mV (n = 252) in good agreement with the literature[2,7]. The inactivation plot is shown in Figure 3 and fit with a Boltzmann equation. The $V_{0.5}$ of inactivation was -75.2 mV (n = 296), also in good agreement with the literature[1,7] when taking into account differences in experimental protocol including $V_{hold}$, pre-pulse duration and ionic composition of solutions.

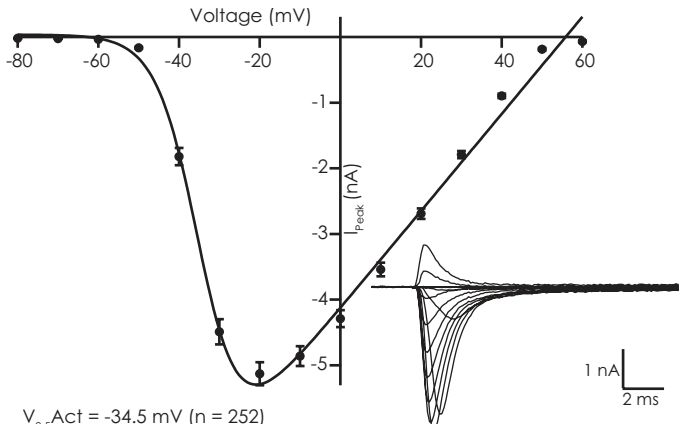


**Figure 2: Current-voltage plot of activation of $Na_V1.5$-ΔKPQ.** The average current-voltage plot for n = 252 wells is shown for $Na_V1.5$-ΔKPQ. The plot is fit with a Boltzmann equation which reveals a $V_{half}$ of activation of -34 mV. The traces for an exemplar well are also shown in the inset.

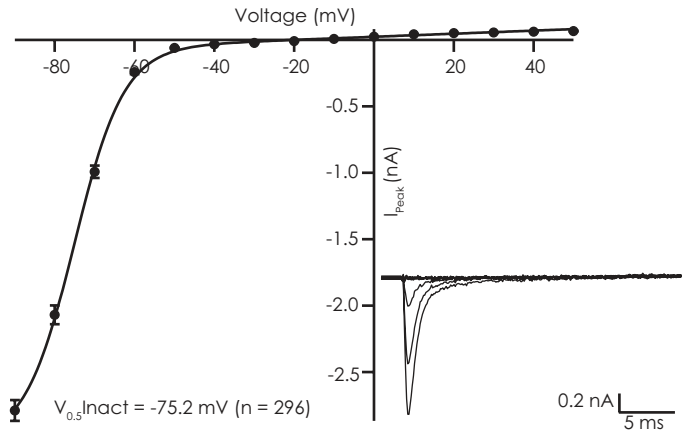


**Figure 3: Current-voltage plot of inactivation of $Na_V1.5$-ΔKPQ.** The average current-voltage plot for inactivation for n = 296 wells is shown for $Na_V1.5$-ΔKPQ. The plot is fit with a Boltzmann equation which reveals a $V_{0.5}$ of inactivation of -75.2 mV. The traces for an exemplar cell are also shown in the inset.

Compounds known to block $Na_V1.5$ were applied to assess their effect on $Na_V1.5$-ΔKPQ peak and late currents. Concentration response curves for mexiletine and ranolazine are shown in Figures 4 & 5, respectively. $IC_{50}$ values are summarized in Table 1. Both mexiletine and ranolazine blocked the late current with a lower $IC_{50}$, i.e. higher potency, than the peak current as expected[6].

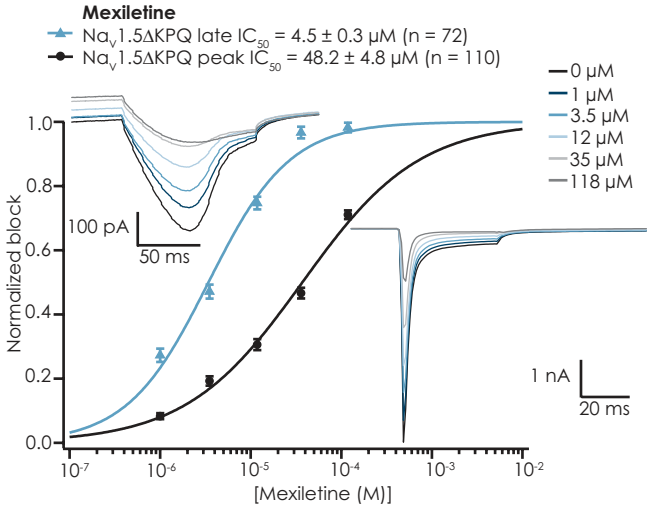


**Figure 4: Inhibition of peak and late currents mediated by $Na_V1.5$-ΔKPQ by mexiletine.** Mexiletine was applied in a cumulative manner from lowest concentration to highest concentration on each well. Concentration response curves for peak current (black circles) and late current (blue triangles) were constructed and are shown for an average of 72 wells (peak) and 110 wells (late) including the average current traces. Multi-hole chips (4 holes per well) were used.

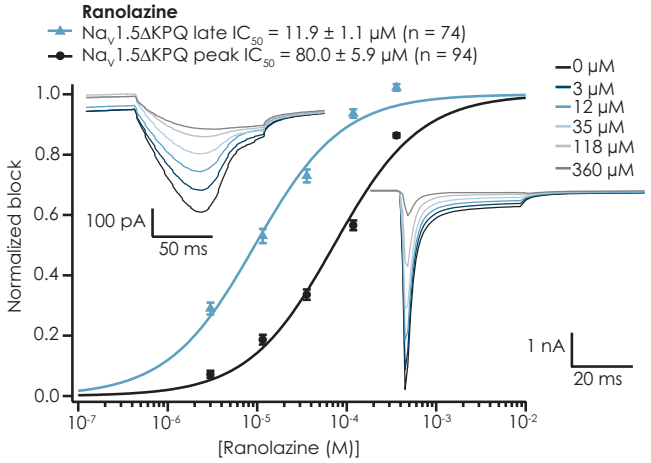


**Figure 5: Inhibition of peak and late currents mediated by $Na_V1.5$-ΔKPQ by ranolazine.** Ranolazine was applied in a cumulative manner from lowest concentration to highest concentration on each well. Concentration response curves for peak current (black circles) and late current (blue triangles) were constructed and are shown for an average of 74 wells (peak) and 94 wells (late) including the average current traces. Multi-hole chips (4 holes per well) were used.

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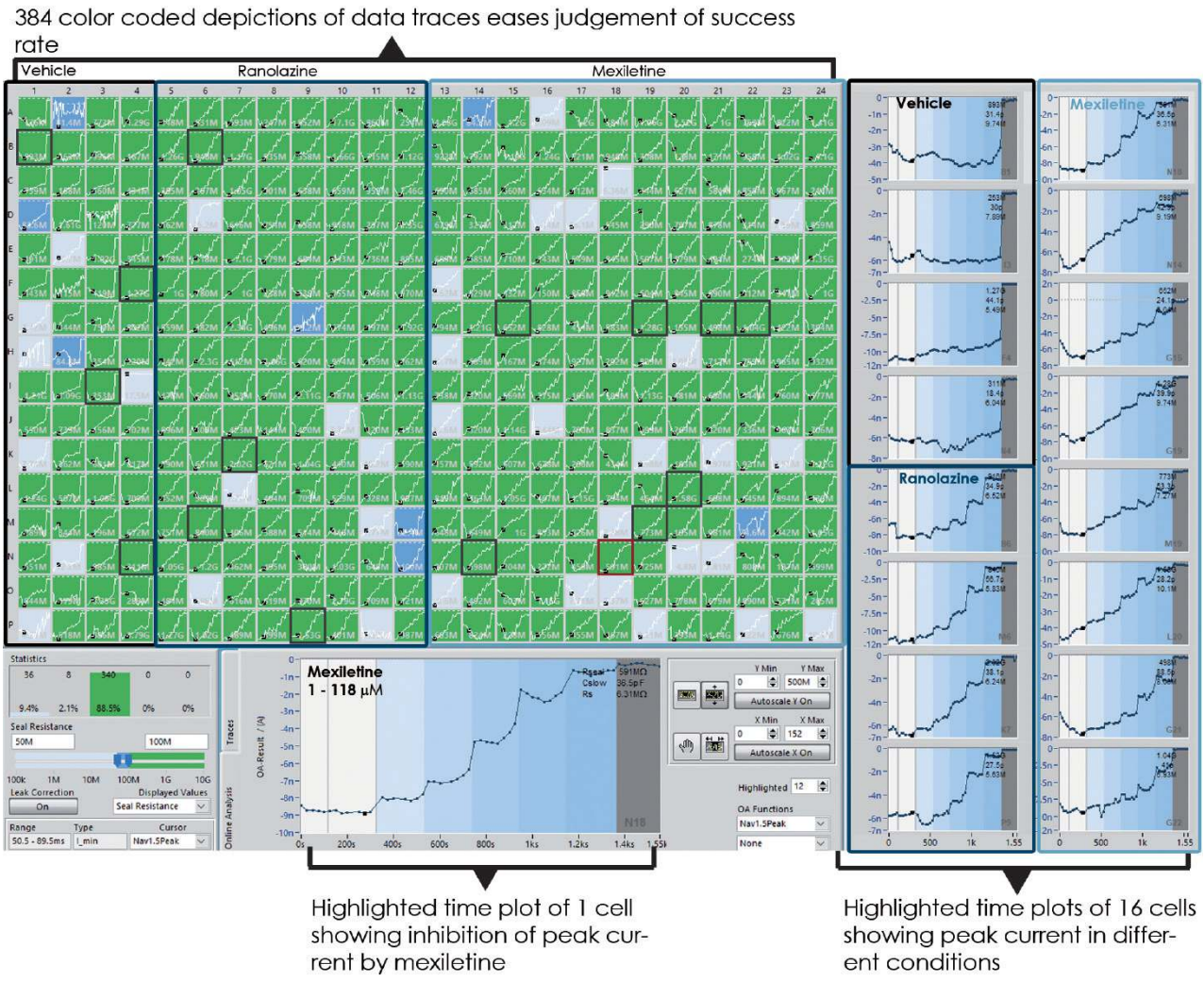


**Figure 6: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384i.** Screenshot depicts online analysis (peak amplitude) of $Na_V1.5$-ΔKPQ-expressing HEK cells as recorded on one NPC-384 patch clamp chip. At the start of the experiment, 1 vehicle application is made followed by 5 concentrations of ranolazine or mexiletine with an incubation time of 3.5 mins per concentration. At the end of the experiment, a full-block concentration of tetracaine (333 µM) was added to each well. As a control, part of the chip received only vehicle. Multi-hole chips were used where 4 holes were present per well. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green (Rmemb > 100 MΩ), blue (Rmemb 50 - 100 MΩ), light blue (Rmemb <50 MΩ) or grey (well disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show online analysis data (peak amplitude) of $Na_V1.5$-ΔKPQ in increasing concentrations of either ranolazine or mexiletine. Using the voltage-step protocol, $IC_{50}$s can be calculated for both peak and late current in each well using a single voltage protocol.

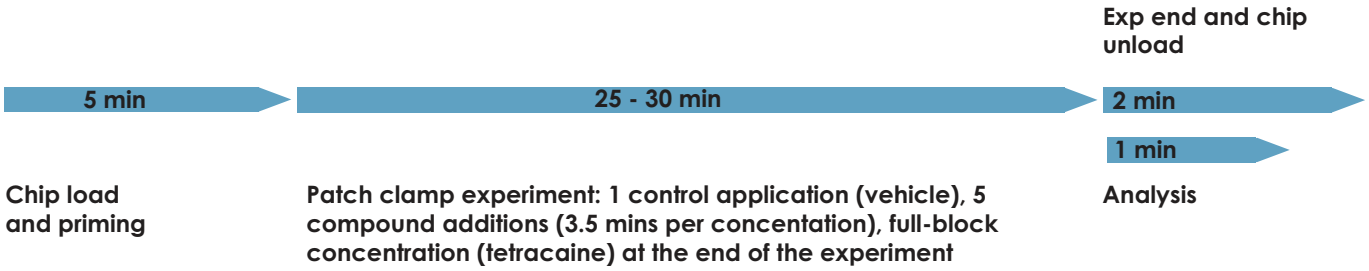


**Figure 7: Timeline of an experiment on the SyncroPatch 384i.** The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a cumulative concentration response curve with control application, cumulative additions of compound and full-block on $Na_V1.5$-ΔKPQ-mediated currents took approximately 32 - 37 mins.

# Application Note

| | $Na_V1.5$-WT $IC_{50}$ (µM) | | $Na_V1.5$-ΔKPQ $IC_{50}$ (µM) | | Literature $IC_{50}$ (µM) | |
|---|---|---|---|---|---|---|
| **Compound** | Peak | Late | Peak | Late | Peak | Late* |
| Ranolazine | 143 ± 9 (63) | - | 80.0 ± 5.9 (94) | 11.9 ± 1.1 (74) | 79.5 ± 5.5 (12) | 16.7 ± 1.7 (5) |
| Mexiletine | 83 ± 5 (83) | - | 48.2 ± 4.8 (110) | 4.5 ± 0.3 (110) | 21.9 ± 2.6 (10) | 12.2 ± 1.1 (12) |

**Table 1:** $IC_{50}$ values for ranolazine and mexiletine block of peak current of $Na_V1.5$-WT (single hole chips) or peak and late current of $Na_V1.5$-ΔKPQ (4 hole chips) and comparison with literature values[6]. * in the presence of ATX-II. Values are given as mean ± S.E.M, number of wells in brackets.

Figure 6 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, online analysis values are chosen where peak amplitude in increasing concentrations of ranolazine or mexiletine are shown. In these experiments, 1 control application of vehicle followed by 5 concentrations of ranolazine or mexiletine in increasing concentrations was applied (3.5 mins incubation per concentration) and finally a full-block application of tetracaine was added. Concentration response curves were calculated for each well. The cells were stable for at least 30 minutes. An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, peak and late $I_{Na}$ could be reliably recorded from $Na_V1.5$-ΔKPQ expressing HEK cells without pharmacological enhancement which could potentially interact with test compounds. Late $I_{Na}$ from $Na_V1.5$-ΔKPQ was more potently inhibited by ranolazine and mexiletine than peak $Na_V1.5$-ΔKPQ or WT peak currents. Using the SyncroPatch 384i automated patch clamp system in combination with the $Na_V1.5$-ΔKPQ cell line provided a reliable high throughput CiPA-compliant cardiac safety screening assay without the need for openers such as ATX-II.

## References


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## Methods

### Cells

HEK cells expressing $Na_V1.5$-ΔKPQ were kindly provided by Metrion Biosciences. CHO cells expressing $Na_V1.5$-WT were also used.

### Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

### Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384i using single hole or multi-hole (4 holes per well) chips. A voltage step to -15 mV was used to record peak current, and a voltage ramp from 40 mV to -80 mV over 100 ms was used to record late $I_{Na}$ current. Holding potential was -80 mV. For pharmacology experiments, compounds were applied in increasing concentrations to each well (incubation time: 3.5 mins per concentration) followed by a full-block concentration of tetracaine (333 µM). Concentration response curves for individual wells were constructed with minimum fixed to 0 and maximum fixed to 1. Average concentration response curves were then constructed for all successful wells for each compound using DataControl 384. Mean and S.E.M was calculated from the individual $IC_{50}$ values. Data was only included in the analysis when it satisfied QC parameters: $R_{seal}$ >400 MΩ, late $I_{Na}$ >-100 pA for single hole chips and $R_{seal}$ >250 MΩ, late $I_{Na}$ >-200 pA for multi-hole chips.


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