The IonWorks Barracuda system enables continuous voltage clamp, from 384 parallel channels continuously and simultaneously, from 384 parallel recording sites. To achieve this, IonWorks Barracuda system is equipped with 384 individual patch-clamp amplifiers together with a 384-channel fluidic pipette. Similar to its predecessor, IonWorks Quatro, IonWorks Barracuda system measures cell membrane currents using the perforated patch clamp techniques on a polylamide substrate. Currents are measured using a single hole at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Friel et al., 2002). In this study, we evaluated a new technique to further prolong the assay window for ionic currents on the IonWorks Barracuda instrument. Using Nav1.5 channel as an example, we demonstrated stable recording of Nav1.5 currents elicited by repetitive scans for 30 minutes or longer. This prolonged assay window, validated using multiple recording parameters, is about twice of that of IonWorks Quatro. This allows the researchers not only to precisely control the state of the channel by voltage clamp, but also to potentially analyze molecules with slow binding rate to the channel. To simulate the work flow in a drug screening, here we designed and validated a single-blind assay to first screen and then confirm use and non-use-dependent blockers of Nav1.5 channels. The high throughput, high sensitivity and the robustness of IonWorks Barracuda system makes it an ideal platform in screening use-dependent channel blockers in a drug discovery environment.

**Material and Methods**

**Cells:** Chinese hamster ovary (CHO) cells stably expressing the human SCN5A (Nav1.5) gene.

**Reagents and buffers:** Amphotericin B (Sigma Cat. # A-4888), DMEM (Sigma Cat. # D-2620); Internal buffer contains (in mM): 100 K gluconate, 10 KOAc, 3.2 MgCl2, 5.0 EGTA, 5.0 HEPES, pH 7.25 with KOH; External buffer is Phosphate Buffered Saline (PBS, Gibco Cat. # 14040).

**Compound management:** All test compounds were obtained from Sigma-Aldrich, and freely prepared everyday following a single-blinded procedure. The final test plate contains 0.1% DMSO.

**Electrophysiology:** All experiments were performed in the PPC mode, with voltage-clamp, and compound addition protocols described in the figure.

**Data analysis:** In addition to the native filter, two data filtering criteria were implemented: 1) seal resistance < 50 MΩ and 2) seal resistance change > 50% (pre vs. post). The data were analyzed and plotted using Phem 5 software.

**Fig. 1.** IonWorks Barracuda™ automated patch clamp system records ion channel currents continuously and simultaneously, from 384 parallel recording sites. A) External view of the system; B) Front view of the process deck.

**Fig. 2.** The voltage-dependent activation and voltage dependence of inactivation of Nav1.5 channels were examined on the IonWorks Barracuda system, in the PPC mode. A) and B) represent representative Nav1.5 currents elicited from one well, in PPC mode; Bottom: zoom-in view of the currents elicited by the first (P1) and last (P30) pulse.

**Fig. 3.** To screen for use and non-use dependent compounds, a train protocol was designed and validated based on the biophysical properties of the channel: A) Top: diagram of the voltage protocol, Vh = -100mV, test voltage = +35mV for 200ms. Middle: representative Nav1.5 currents elicited from one well, in PPC mode; Bottom: zoom-in view of the currents elicited by the first (P1) and last (P30) pulse. B) Description of the screening process: after baseline measurement, in each assay 10µl compound (2X concentration) or buffer was introduced into PatchEPL well at medium position, with no mixing. Three post-compound scans were collected at 0s, 90s, and 180s after compound addition. C) The current amplitudes of the last pulse at 4 and 180s, for all scans, were examined by repeated measures one-way ANOVA data, and compound efficacy (use and non-use dependent) was identified among all groups, indicating the stability of currents over-time, and after compound (buffer in this case) addition.

**Fig. 4.** Evaluation of assay window for Nav1.5 channels, and recording stabilizes among multiple experiments. A) Post-screening of 30-minute high voltage clamp window, and at different time points. The pharmacology data collected on IonWorks Barracuda system is in good agreement with literature reports.

**Fig. 5.** Screening for use (lidocaine and tetracaine) and non-use dependent (phenolamine, TTX) blockers for Nav1.5 channels. A) plate map of the single point compound plate, with 10µM final concentration in 0.1% DMSO. In circles are representative Nav1.5 currents elicited from the same well (top traces), using different voltage protocols (bottom); B) and D) the current-voltage relationships for both activation and inactivation of Nav1.5 channels, data was collected from one experiment (mean ± SEM, n= 382 wells, 2 wells filtered out).

**Fig. 6.** Pharmacological characterization of concentration-response relationship, for confirmation of screening hits. A), plate view of Nav1.5 currents (pulse 30 only) in response to different concentrations of compounds (TTX, lidocaine, tetracaine) or buffer; B), heat map view of the wells with more than 50% inhibition of pulse 30 currents; C), representative currents (top) and the overlay of pre- and post-compound currents (bottom) from the same well.

**Fig. 7.** Identification of use-dependent compounds by comparing concentration-response curves. Analysis of use dependent pharmacology A) and pulse 30 B), and at different time points. C) data scatter from three different experiment, indicating highly consistent results.

**Validation of Nav1.5 screening protocol**

**Summary**

- In this study we designed and validated a high-throughput electrophysiology assay for screening and confirming blockers of a voltage-gated sodium channel. The pharmacology data collected on IonWorks Barracuda system is in good agreement with literature reports.
- The IonWorks Barracuda system enables continuous voltage clamp, optimized voltage protocols, flexible recording parameters, and prolonged assay window, for identification of use-dependent compounds in a drug screening setting.