Avoid the Fear of Missing Out: Early, Thorough Assessment of Assay Translation is Crucial in Dictating Screening Strategy and Reducing the Risk of False Negatives

Oscar Dew[1], Merilyn M. Albuquerque[1], Mateusz Kurpiewski[1], Christopher A. Hinckley[2], Sanjay Danthi[2], Melissa Bennion[2], Gareth W. Langley[1]
[1]Charles River Laboratories, Chesterford Research Park, CB10 1XL, United Kingdom
[2]Biogen, 225 Binney Street Cambridge, MA 02043

charles river

1 INTRODUCTION

- Advances in the automated patch-clamp techniques have made HTS campaigns for ion channels using these platforms feasible[1].
- However, automated patch-clamp assays are more costly and associated with longer timelines than traditional fluorescence-based assays.
- An attractive HTS strategy is to use a fluorescence-based assay as the primary screening method and follow up with a patch-clamp assay[2].
- This approach can help rapidly progress through a HTS campaign; however, it is crucial that the primary assay translates to the functional readout which is used to triage hit material.

Key Findings

In side-by-side comparison of two commercially available assay kits, despite similar assay performance, test compounds exhibited in different response profiles.

Automated patch-clamp studies revealed that one of the assay kits was masking the activity of active compounds.

2 RESULTS

Similar Headline Performance and Control Compound Response

Molecular Devices Potassium Assay

Average Robust Z' = 0.64

Average S:B = 9.93

Thermo FluxOR II Potassium Ion Channel Assay

Average Robust Z' = 0.55

Average S:B = 6.77

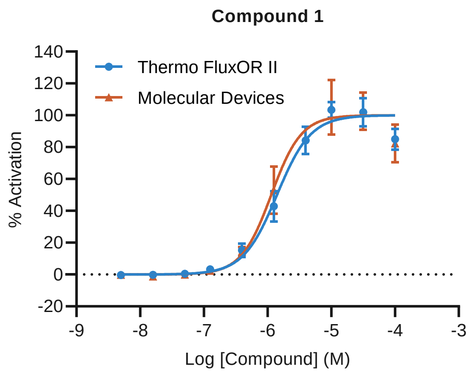
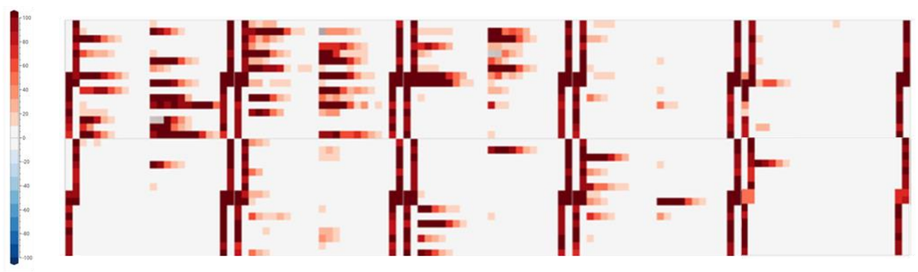


Figure 1. Fluorescence-based assay performance of two assay kits and CRC analysis of a commercially available control (Compound 1, n=3). S:B = signal-to-background ratio.

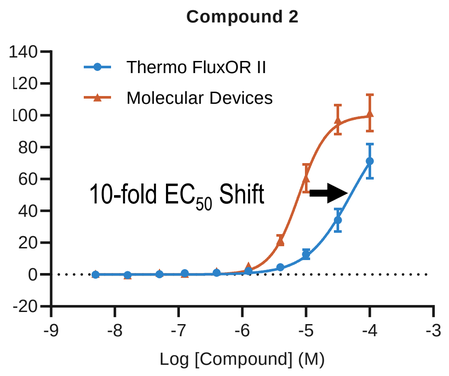
Different Behaviour Observed With a Wider Range of Test Compounds



Pilot Screen Hit Rates:

Molecular Devices: **0.22%**

Thermo FluxORII: **0.09%**



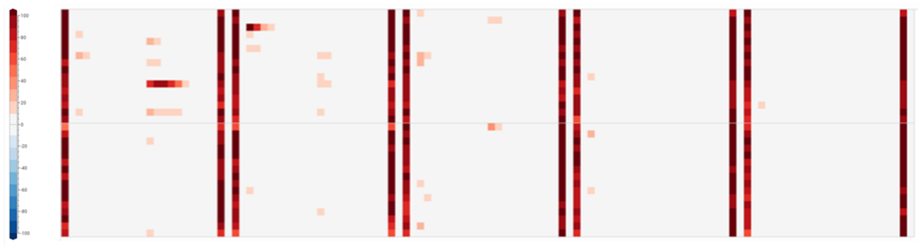
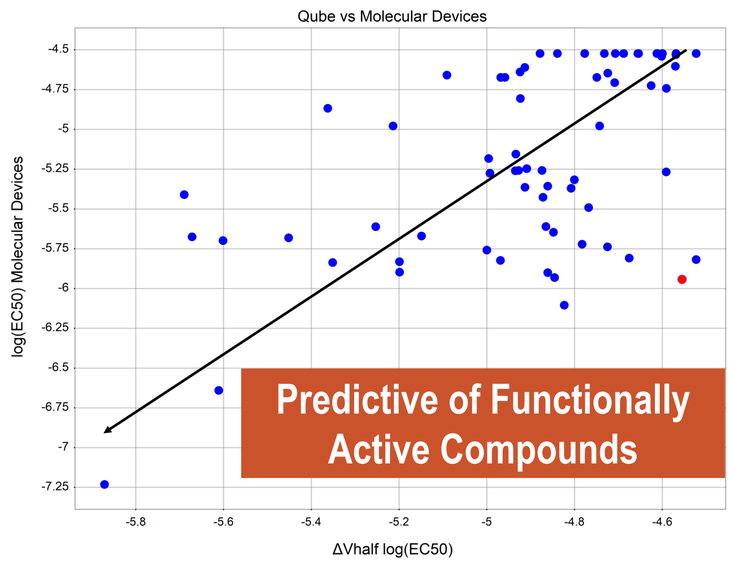


Figure 2. Stark differences were noted between the two kits when work progressed to the pilot screen. This difference continued when screening proprietary compounds in CRC format (heat maps shown) and a commercially available K^+ channel activator, Compound 2 (n=3). All screening was performed on a FLIPR Penta.

Predictive of Functional Readout?



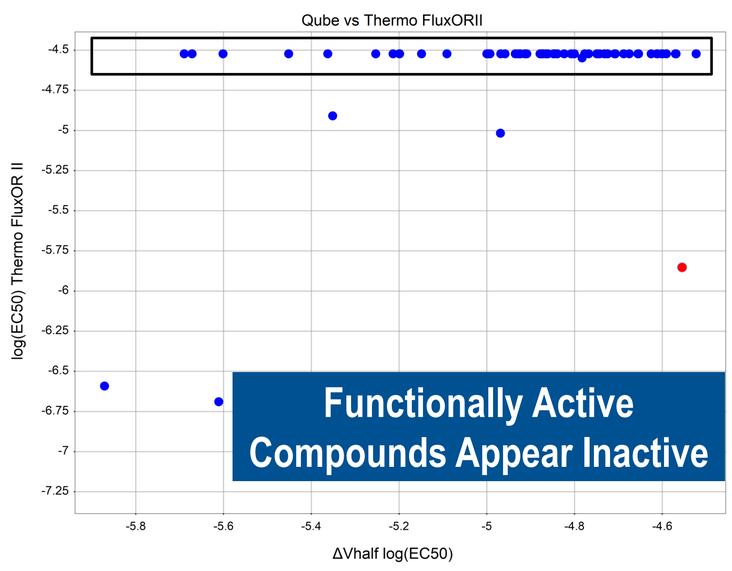


Figure 3. Scatter plot of hit compounds validated on the Sophion Qube 384 against each assay kit. The positive control (Compound 1) is highlighted in red.

3 SUMMARY AND CONCLUSIONS

- Despite similar performance of the Thermo FluxOR II and Molecular Devices assay reagents during assay development, drastically different results were seen in the pilot screen and for a compound subset. Follow up activities revealed functionally active compounds were not detected by the Thermo FluxOR II reagents.
- Some compounds performed similarly across both assays, suggesting that the effect is not uniform. Therefore, choosing assay reagents based on only one or two compounds could not only result in false negatives, but highly mislead downstream hit triage and SAR determination.
- This study once again highlights the importance of thorough assay validation, particularly if translation to a functional readout is required, in order to increase the likelihood of identifying high quality hit material from an HTS campaign.

4 ACKOWLEDGMENTS

The authors would like to thank all members of the project teams at both CRL and Biogen for their help and support throughout. In particular, we would like to acknowledge the support of Gary Clark, Sarah Williams, Scott Maidment, Juha Kammonen, Param Murugan and Richard Nelson. We would also like to thank Joshua Betts, Harry Hickey, Thomas Hill and the CRL tissue culture team for practical support in the execution of this project.

5 REFERENCES

1. Obergrussberger, A. Friis, S., Bruggemann, A. Fertig, N. *Expert Opinion on Drug Discovery*. 2020; **16:1**, 1-5
2. Yue, J., Qiao, G., Liu, N., Nan, F. and Gao, Z. *Nature*. 2016; **37**, 105-110