LifeArc

Development of a novel screening system to identify activators of Two-pore domain potassium channels (K2Ps)

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OVERVIEW

- **Aim: Develop assays capable of identifying novel activators of K2P channels**
- **U-2 OS cells transduced with BacMam to generate cells expressing functionally active K2P channels**
- **K2P function measured using thallium flux**
- **LifeArc Index set (11k compounds) screened**
- **Selectivity and potency of activators investigated**

INTRODUCTION

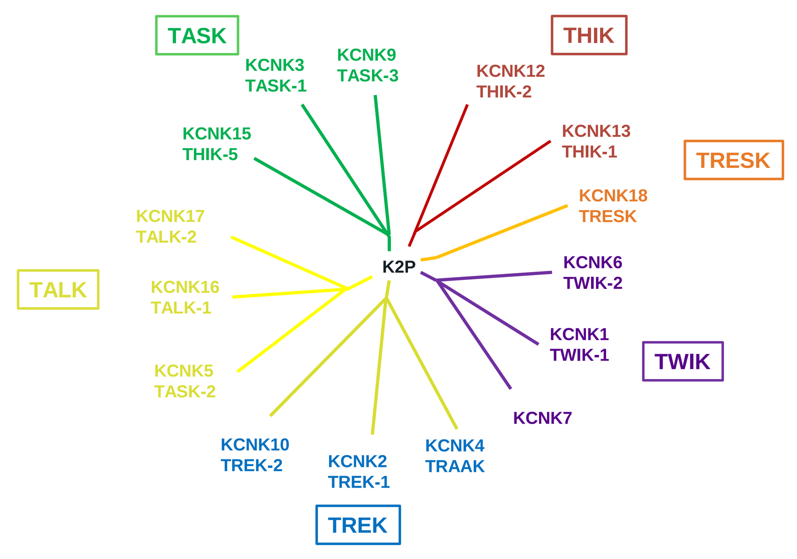


Figure 1 - K2P superfamily

K2P channels are characterised by their four transmembrane domain, two-pore topology. Sub-units dimerise to form a functional channel.

K2P channels carry background (or leak) potassium current in a variety of cell types and primarily act to maintain resting membrane potential.

Despite a number of important roles they have proved a difficult target class to modulate with small molecules.

Thus, there is currently a lack of useful specific pharmacological tools which target K2Ps. This has limited the interrogation of the precise physiological function of K2Ps and efforts to generate K2P targeting therapeutics.

K2Ps have been implicated in a number of human pathophysiologies including, but not limited to, pain (TREK-1, TREK-2, TRAAK), migraine (TRESK), Birk-Barel syndrome (TASK-3) and Balkan endemic nephropathy (TASK-2).

FUNCTIONAL EXPRESSION OF K2P CHANNELS

Generation of cell lines stably over-expressing ion channels can be challenging and the ability to identify channel activators can be compromised in systems where the target is expressed at high levels. To avoid these issues we used baculovirus (BacMam) to deliver K2P channels into mammalian cells. BacMam allows the precise titration of expression of the gene of interest. This enables cell systems to be generated in which we can intricately and robustly select a level of K2P expression, (via function) optimized for the identification of activators.

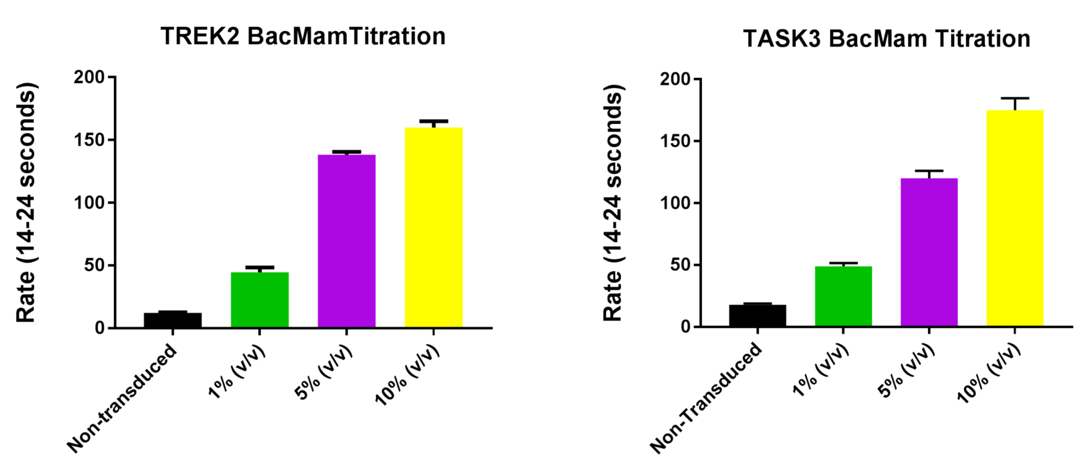


Figure 2 -Titratable expression of functional channel using BacMam. Graphs show rate of fluorescence increase using thallium flux as a measure of channel activity.

SCREENING OF THE LIFEARC INDEX SET

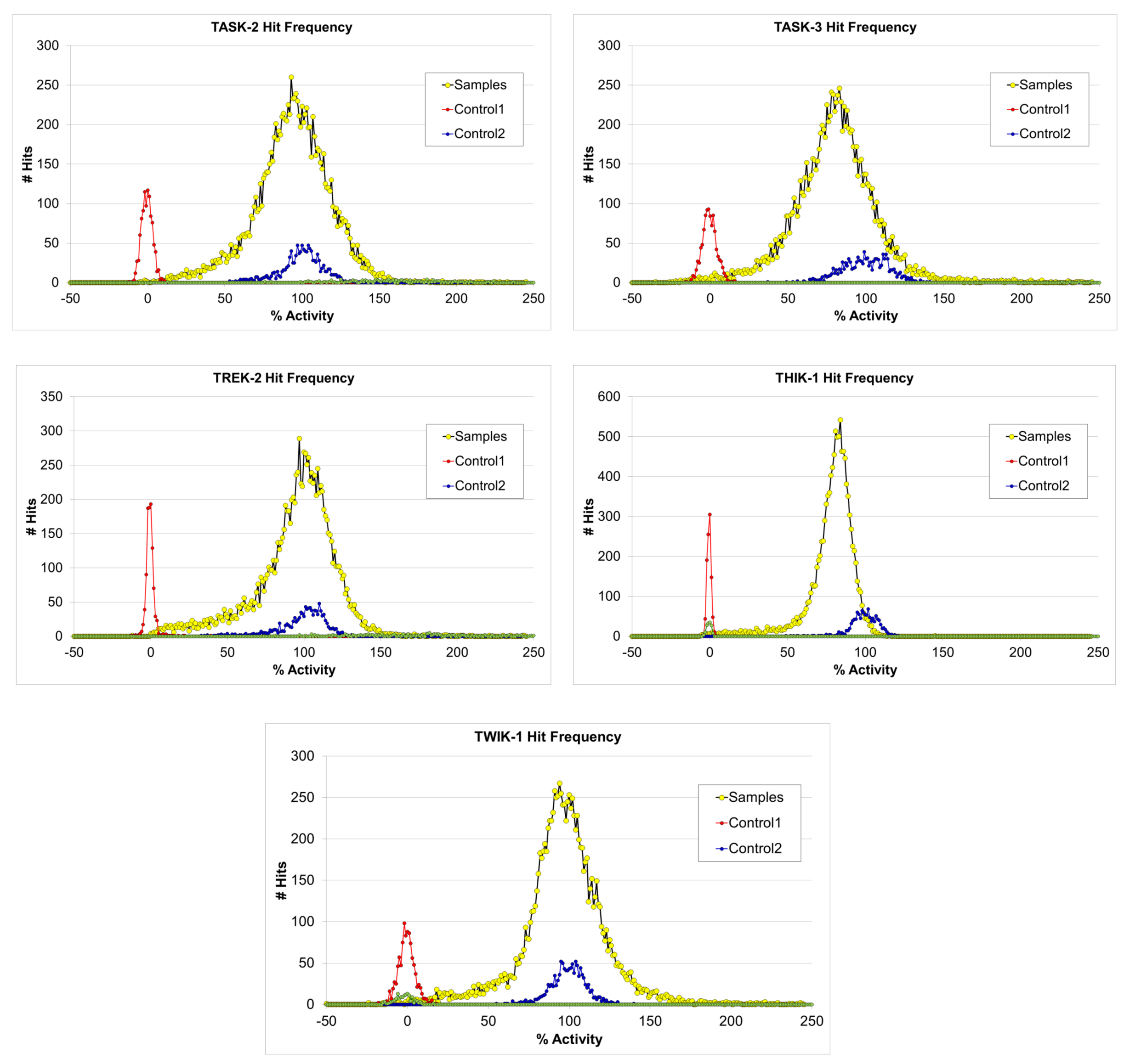
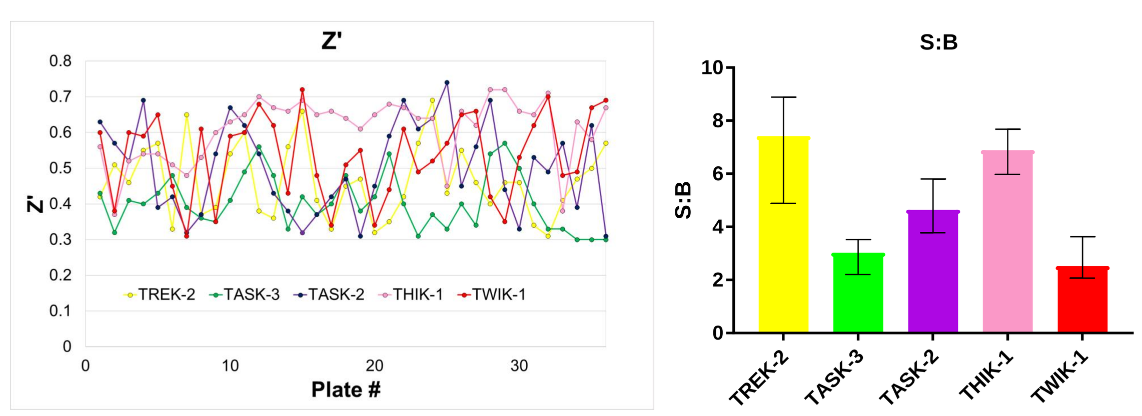


Figure 3 – Summary of K2P thallium flux data. Graphs show number of compounds at each % Activity. 'Samples' denotes 11k compounds of LifeArc Index collection. 'Control 1' is low control (inhibitor), 'Control 2' is high control (DMSO). All responses calculated relative to low (0)% and high (100%) controls.

SCREENING STATISTICS



K2P	Activity cut-off	#Hits	Hit Confirmation
TASK-2	149%	113	43/113
TASK-3	160%	111	80/111
TWIK-1	169%	114	79/111
TREK-2	220%	120	85/120
THIK-1	135%	6	2/6

Figure 4 – Summary of Screening statistics. (Top Left) Z' values for each plate screened across all targets. (Top right) Signal to background ratio (S:B) for each target. Values calculated from low (inhibitor) and high (DMSO) controls. Error bars denote range. (Bottom) For each screen the number of compounds for progression was set at ~1% , giving a variable activity cut-off. Hit confirmation n=2.

MATERIALS AND METHODS

U-2 OS cells were transduced with varying concentrations (v/v) of BacMam (SB Drug Discovery): TREK-2 (1%), TASK-3 (5%), THIK-1 (0.05%), TASK-2 (1%). In each case frozen cells were thawed and BacMam solution added before cells were plated at 5k cell per well and incubated overnight at 37°C with 5% CO_2. For TWIK1, U-2 OS cells were plated in a T-175 flask with 10% BacMam for 1hr. Cells were incubated in fresh media in flask overnight before being plated at 5k per well and incubated for a further 24hrs.

Channel function was measured using FLIPR Potassium kit (Molecular Devices, USA) as per manufacturers guidelines.

Activity was calculated as rate of fluorescence increase after thallium addition.

Compounds screened at 10μM final assay concentration.

SELECTIVITY

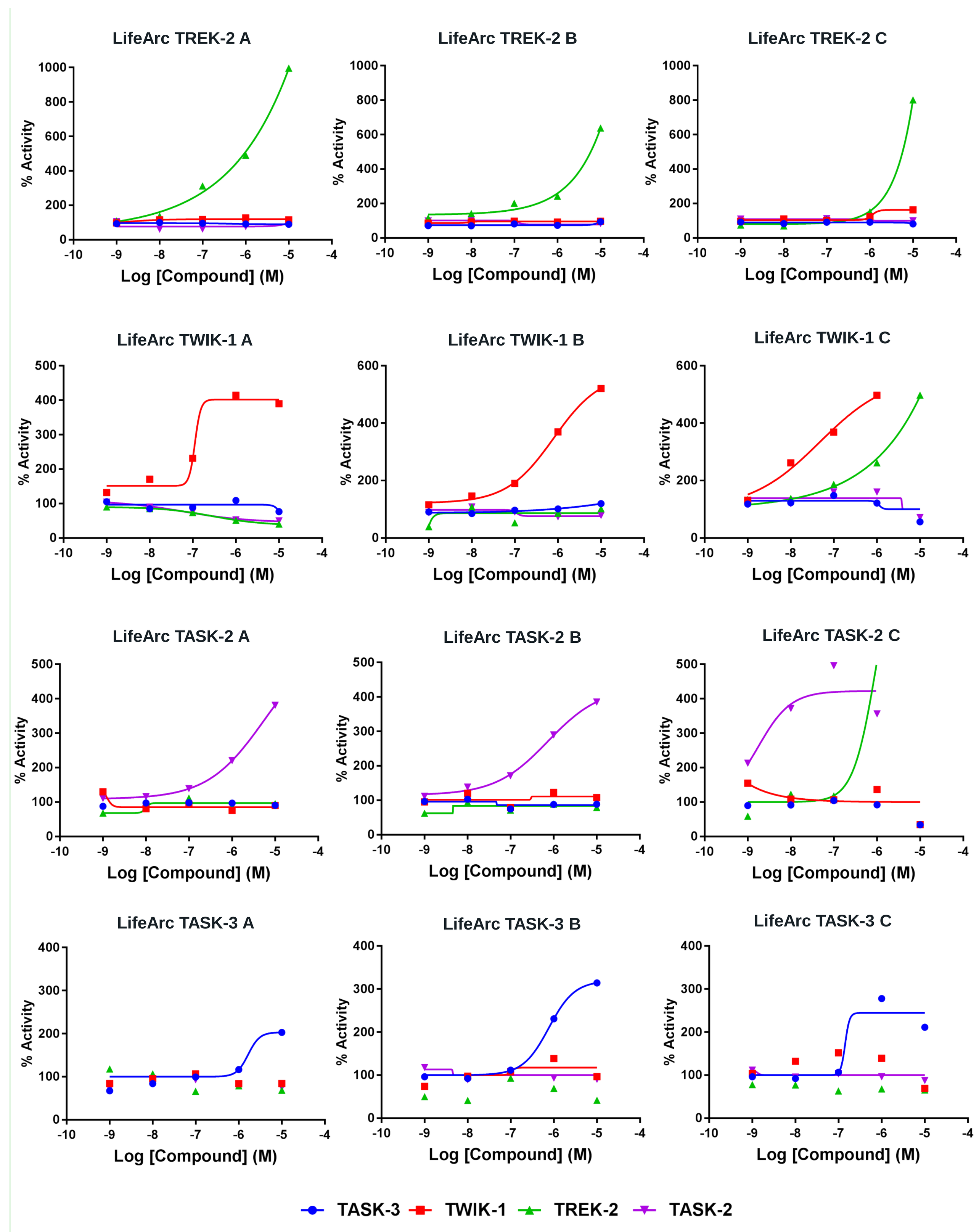


Figure 5 – Representative 5 point curves of hit compounds. For hit confirmation and initial selectivity observations all 'hit' compounds were analyzed at 5 concentrations versus 4 K2P channels. Compounds were prepared using a 'through plate' dilution method. Graphs show % activity using rate of fluorescence increase in thallium flux assay as a measure of channel activity. Graphs show single compound at multiple channels.

POTENCY

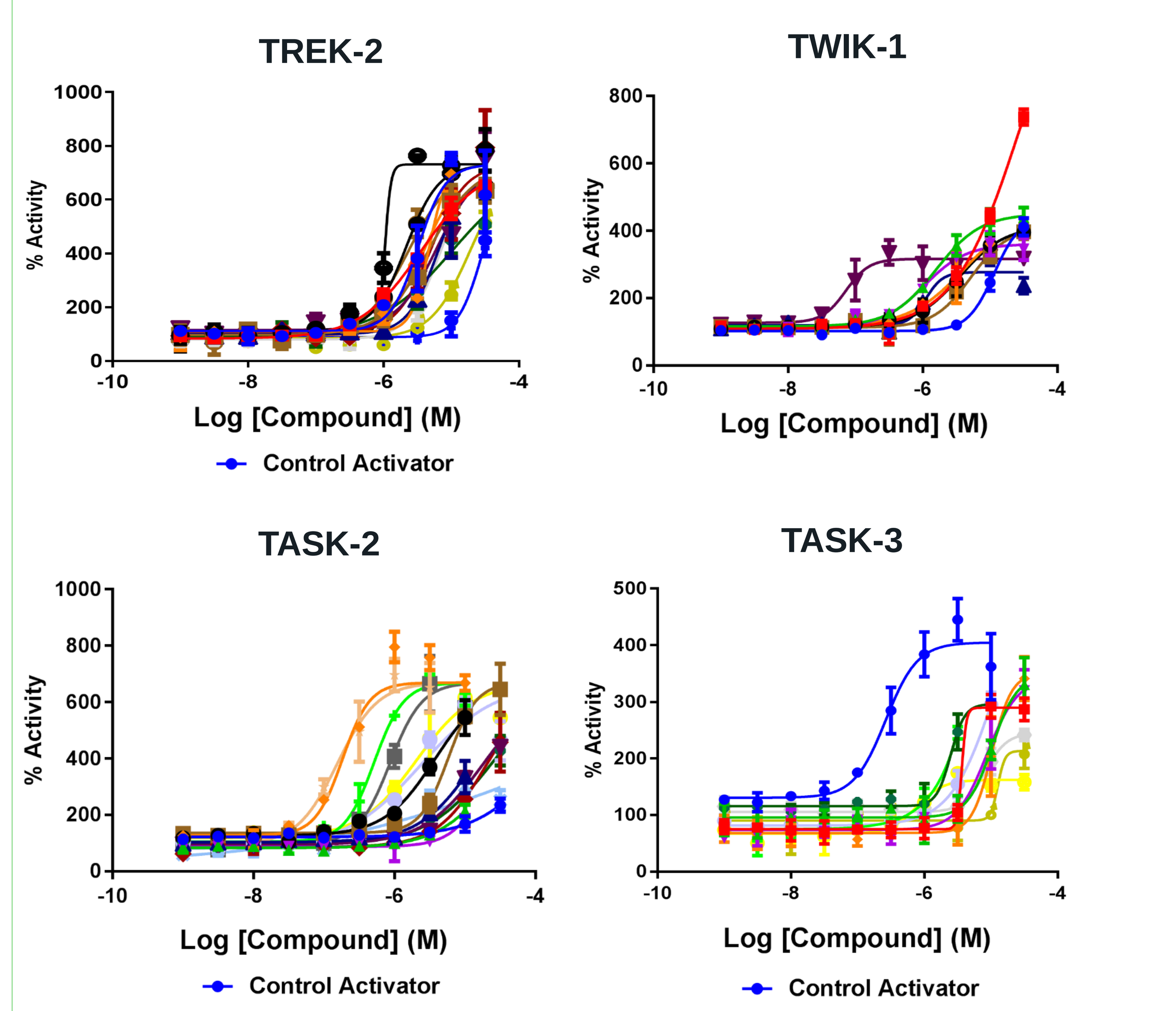


Figure 6 – Representative 10 point curves of lead compounds. After chemistry triage and selectivity analysis 'hit' compounds were analyzed using 10 point compound response curves. Graphs show % activity using rate of fluorescence increase in thallium flux assay as a measure of channel activity. Graphs show multiple compounds at a single channel. Data is n≥2, +/- standard deviation.

Conclusions

Assays developed using titratable expression

Allowed identification of novel K2P activators

Activators show selectivity at K2P channels

Not all K2Ps 'druggable' using assay system described

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