LifeArc

Identification of novel activators of two-pore domain potassium (K2P) channels
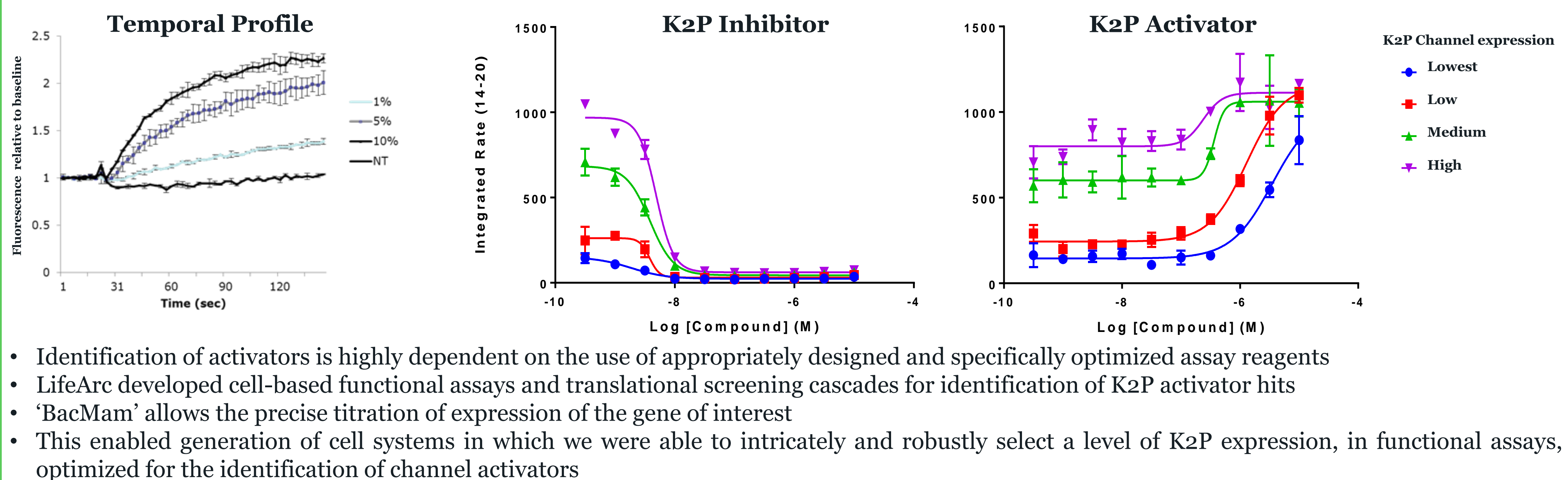
David McCoull, Lewis Byrom, Jonathan M Large, Jeff Jerman, Paul D Wright

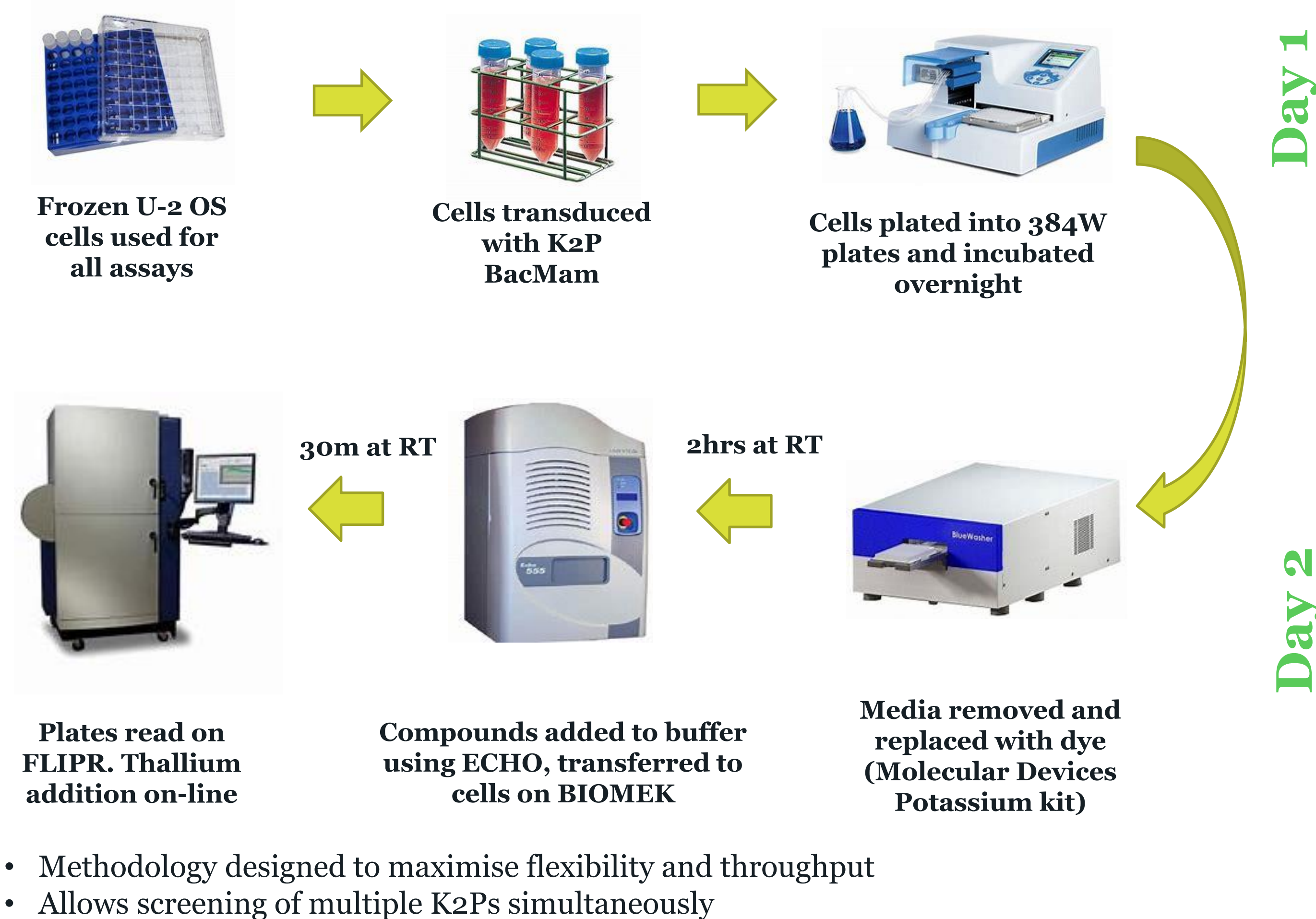LifeArc, Accelerator Building, Open Innovation campus, Stevenage, SG1 2FX, United Kingdom

OVERVIEW

- Two-pore domain potassium channels (K2Ps) carry background (or leak) potassium current
- Primarily act to maintain resting membrane potential.
- K2P channels are characterised by their four transmembrane domain, two-pore topology
- Genetic and functional evidence points to a role in multiple pathophysiologies, including pain and migraine
- K2Ps have proved difficult to modulate with small molecules and there is a lack of useful specific pharmacological tools
- This has limited the interrogation of their precise physiological function and efforts to generate K2P based therapeutics
- LifeArc developed a novel system to identify K2P activators, with the aim of providing tools for research and ultimately novel therapeutics
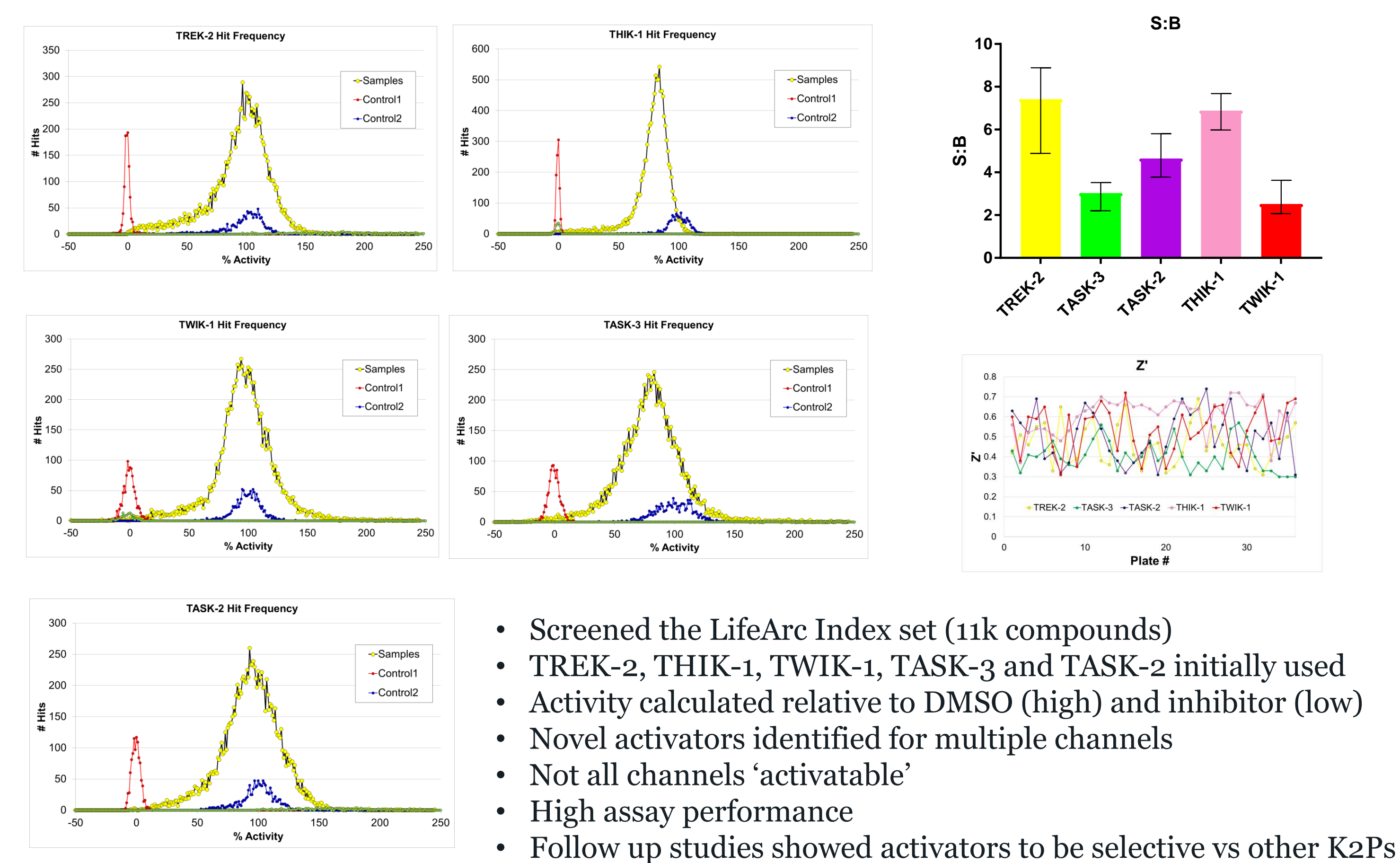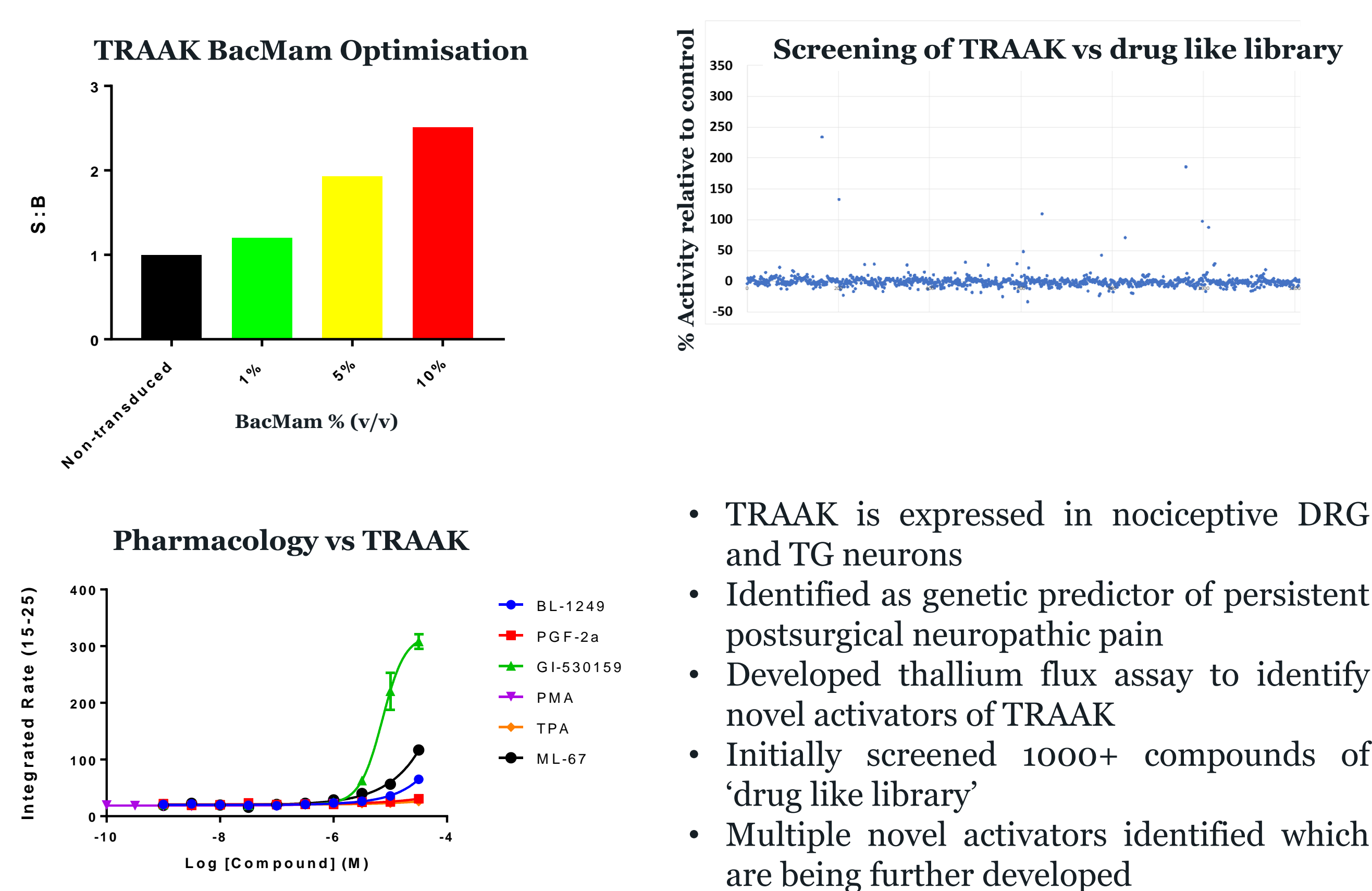
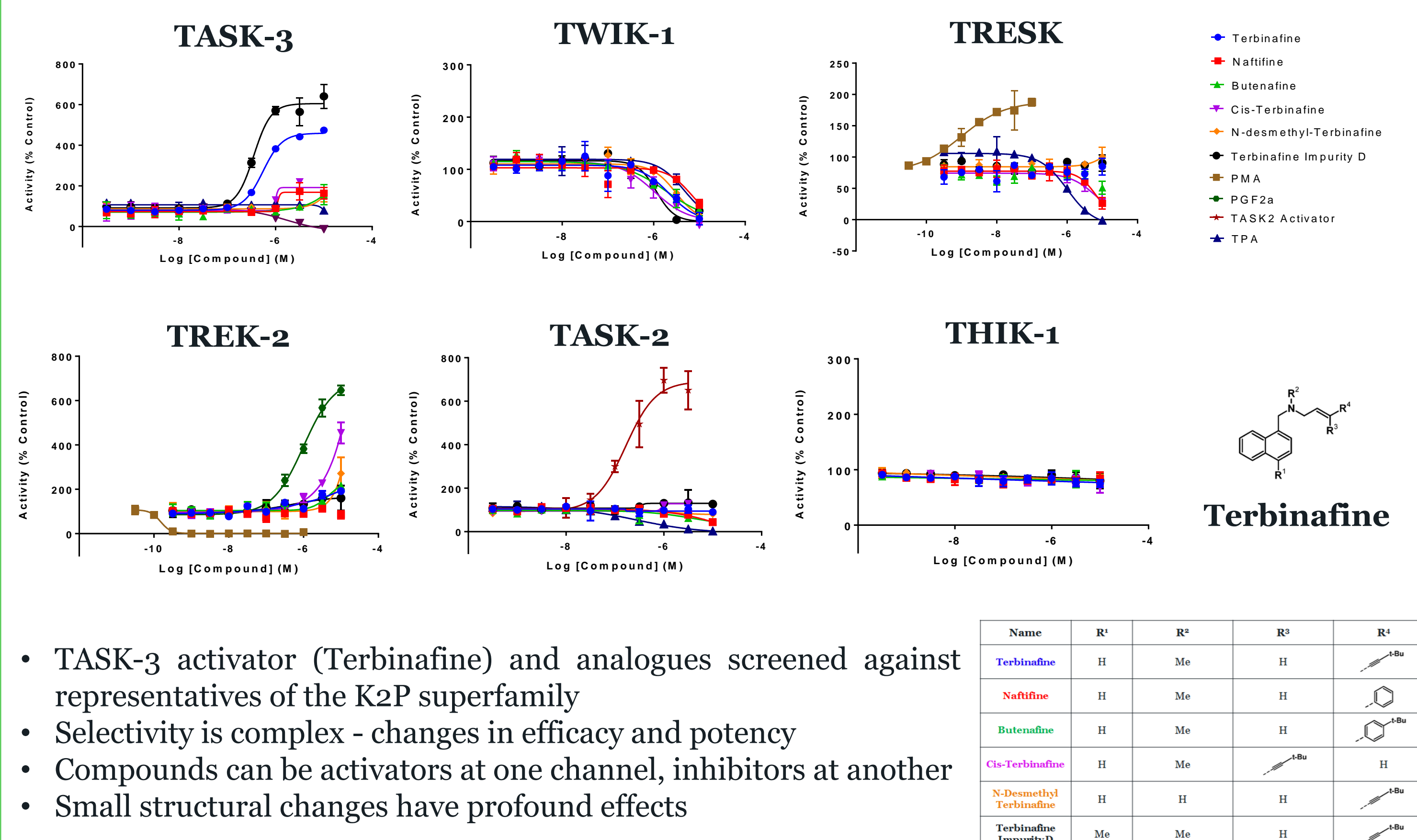## IDENTIFYING ACTIVATORS REQUIRES BESPOKE ASSAYS/REAGENTS

- Identification of activators is highly dependent on the use of appropriately designed and specifically optimized assay reagents
- LifeArc developed cell-based functional assays and translational screening cascades for identification of K2P activator hits
- 'BacMam' allows the precise titration of expression of the gene of interest
- This enabled generation of cell systems in which we were able to intricately and robustly select a level of K2P expression, in functional assays, optimized for the identification of channel activators

ASSAY PROCESS AND METHODS

Frozen U-2 OS cells used for all assays

Cells transduced with K2P BacMam

Cells plated into 384W plates and incubated overnight

Day 1

Day 2

Media removed and replaced with dye (Molecular Devices Potassium kit)

30m at RT

2hrs at RT

Plates read on FLIPR. Thallium addition on-line

Compounds added to buffer using ECHO, transferred to cells on BIOMEK

- Methodology designed to maximise flexibility and throughput
- Allows screening of multiple K2Ps simultaneously

SCREENING FOR K2P ACTIVATORS

- Screened the LifeArc Index set (11k compounds)
- TREK-2, THIK-1, TWIK-1, TASK-3 and TASK-2 initially used
- Activity calculated relative to DMSO (high) and inhibitor (low)
- Novel activators identified for multiple channels
- Not all channels 'activatable'
- High assay performance
- Follow up studies showed activators to be selective vs other K2Ps

DEVELOPMENT OF A NOVEL TRAAK ASSAY

- TRAAK is expressed in nociceptive DRG and TG neurons
- Identified as genetic predictor of persistent postsurgical neuropathic pain
- Developed thallium flux assay to identify novel activators of TRAAK
- Initially screened 1000+ compounds of 'drug like library'
- Multiple novel activators identified which are being further developed

SELECTIVITY OF K2P ACTIVATORS

Terbinafine

- TASK-3 activator (Terbinafine) and analogues screened against representatives of the K2P superfamily
- Selectivity is complex - changes in efficacy and potency
- Compounds can be activators at one channel, inhibitors at another
- Small structural changes have profound effects

Name	R¹	R²	R³	R⁴
Terbinafine	H	Me	H	
Naftifine	H	Me	H	
Butenafine	H	Me	H	
Cis-Terbinafine	H	Me		H
N-Desmethyl Terbinafine	H	H	H	
Terbinafine Impurity D	Me	H	H	

CONCLUSIONS

- Assays developed to assess 'ligandability' and facilitate the identification of novel activators of K2P channels
- LifeArc Index set screened and novel activators of multiple K2P channels observed
- Activators show selectivity across K2P channels but selectivity and SAR are complex
- Not all K2Ps are 'druggable' using assay system described