Label free technology: value added information in the drug discovery process

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Introduction

We have evaluated Molecular Devices CellKey™ and SRU BIND® label free detection technologies and their application to hit finding and hit validation using cell based assays. The presented data highlights:

- native cells profiled using a panel of agonists and showing kinetic responses that were characteristic of the published coupling mechanisms of the endogenous receptors
- comparable pharmacology of reference compounds obtained between the label free detection platforms and traditional screening formats, such as calcium flux and htrf
- confirmation of compound activities initially identified in traditional hit finding programs, using cells stably expressing the target receptor

Kinetic responses to endogenous receptors

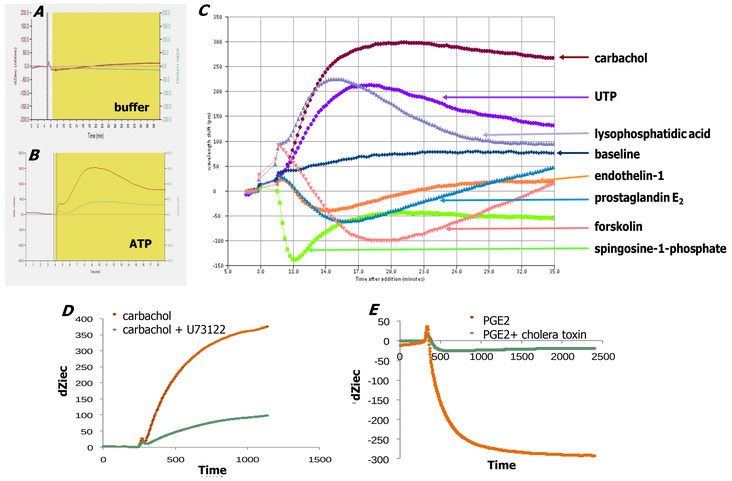
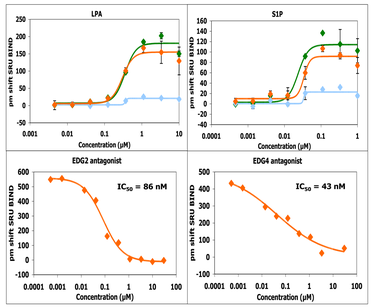


Figure 1. GPCR stimulation evokes different response profiles to different ligands
HEK-293 cells reveal discrete profiles on CellKey (A, B) and SRU BIND (C) when stimulated with agonists of receptors that signal via different G-protein mediated pathways. The neuroblastoma cell line, SHSY5Y, shows an increase in impedance upon activation with carbachol which can be partially blocked with the PLC inhibitor U73122 indicating calcium or $G\alpha_q$ dependence (D). Visually the fingerprint could be interpreted as $G\alpha_i$, highlighting the importance of using pathway modulators. CHO cells treated with PGE2 show a profile indicating activation of an endogenous $G\alpha_s$ receptor which can be blocked with cholera toxin (E).

In HEK cells, S1P gives a profile commonly associated with $G\alpha_{12/13}$ coupling whilst that observed with LPA indicates $G\alpha_i$ coupling comparable to that observed in CHO cells (Figure 2). Conversely, in CHO cells, S1P response is inhibitable with pertussis toxin indicating the response is mediated by $G\alpha_i$. This highlights the need to consider carefully the choice of host cell when designing an over-expression system and to fully understand the coupling mechanism of endogenous receptors in that system.



The response to S1P and LPA treatment can be modulated by pertussis toxin (blue), but not by BAPTA (green), indicating coupling through $G\alpha_i$ proteins. The response to LPA can be inhibited in a concentration-dependent manner with EDG2 or EDG4 inhibitors indicating that both recetor isoforms are endogenously expressed. The IC_{50} values are comparable to those obtained in a Ca^{2+} flux assay (90 nM and 50 nM respectively).

Figure 2. Endogenous LPA-mediated signalling can be studied in wild type CHO cells on SRU BIND

Pharmacology comparison across platforms

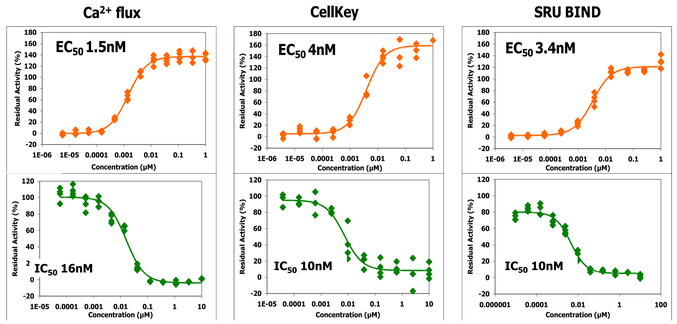


Figure 3. Comparable pharmacology between calcium flux and label free detection
CHO cells stably transfected with a $G\alpha_q$ coupled GPCR were used as a tool to compare pharmacology between assay platforms. Despite the different measurement principles of the three platforms, the EC_{50} of the natural ligand and IC_{50} of a specific inhibitor were found to be comparable.

HEK cells stably transfected with a $G\alpha_i$ coupled GPCR and promiscuous $G\alpha_{16}$ protein were assayed in suspension or as adherent cultures. The EC_{50} of the ligand was largely comparable between assay formats for the two platforms.

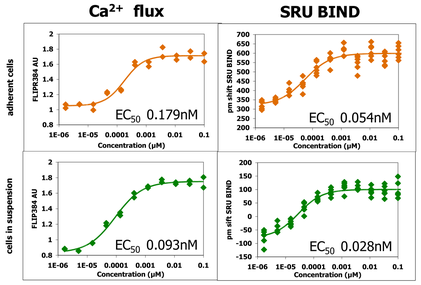
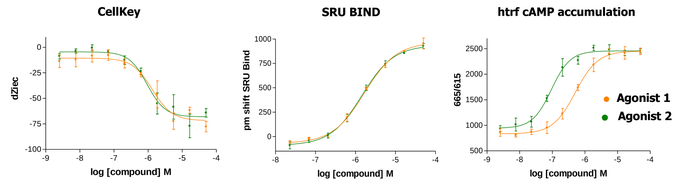


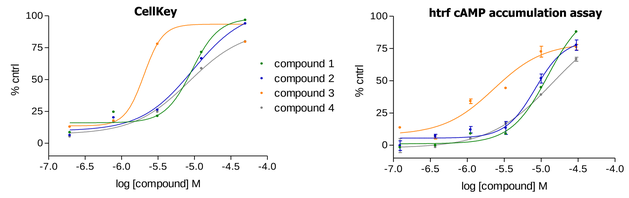
Figure 4. Pharmacology remains comparable when cells assayed as suspension or adherent cultures



Agonist	Assay Platform (EC_{50} M)		
	CellKey	SRU BIND	htrf cAMP
1	1.30E-06	1.70E-06	5.00E-07
2	9.40E-07	1.50E-06	9.40E-08

Figure 5. Differences in pharmacology between label free platforms and a second messenger assay
The potency of two natural ligands were compared across three assay platforms in CHO cells stably transfected with a $G\alpha_s$ coupled GPCR. They were found to be comparable between the two label free systems. However, the data is less comparable with that from an htrf cAMP accumulation assay. In the second messenger assay, the potency of the two agonists can be resolved, in line with expectations from the literature.

Validation of screening hits



Compound ID	Assay Platform (EC_{50} μM)	
	CellKey	htrf cAMP
1	9.2	12
2	9.5	8.3
3	2	2.3
4	8.4	14

Figure 6. Selective hits from HTS can be validated on CellKey
Four compounds from the BioFocus screening collection that were identified as selective hits in an HTS screening campaign were tested on CellKey using the CHO-GPCR cell line employed in the screen. The pharmacology was found to be within three-fold of the original htrf data as shown in the table.

Conclusions and remarks

- label free technology allows the study of endogenous receptors making validation of screening hits on more disease relevant cell lines applicable
- the additional insight into potential signal transduction mechanisms gained from label free detection adds value compared to existing screening technologies. This is particularly informative when characterizing small molecule hits that emerge from HTS or prioritizing active series within hit to lead programs
- comparable pharmacology between platforms could facilitate transition to a label free system for post-screening SAR studies