Label free technology: the weird and wonderful world of cell signaling

Ryall A†, Shearer J†, Brodbeck D*, Nufer O*, Cronk D† and Fasler S*
†BioFocus, Chesterford Research Park, Saffron Walden, CB10 1XL, UK
*BioFocus, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland email: info@glpg.com

Introduction

Whilst conducting an evaluation of label free technology platforms we encountered a number of surprising, but reproducible, observations. For example, receptor over-expression impacts endogenous signaling responses; surface coatings affecting cell adherence can change the response profile; and it is important to choose the appropriate analysis metric for complex profiles. Awareness of the subtleties of cell signaling within model systems such as CHO and HEK recombinant over-expression systems is essential for understanding how small molecules interacting with the pathways are exerting their effects.

Over-expression impacts endogenous signaling

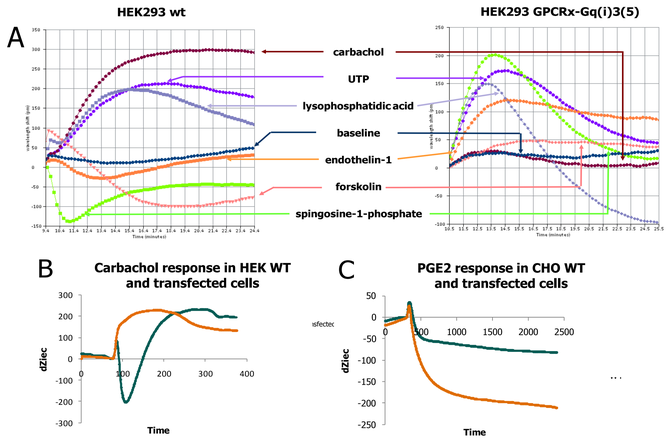
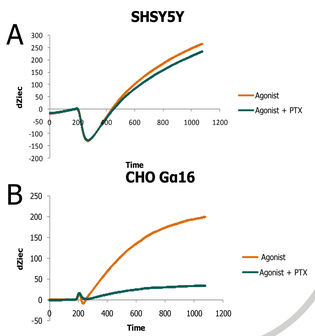


Figure 1. Endogenous signaling is disrupted by over-expression of a GPCR
The response to a natural ligand was examined on SRU's BIND® platform in both wild type HEK-293 cells and the same cell stably expressing a specific GPCR in combination with $G\alpha_{q(i)3(5)}$ protein (A). Stable transfection of cells can have a significant effect on cellular responses to different stimuli. Cases were observed where stimulation was completely abolished (e.g. carbachol), or different profiles were elicited (e.g. sphingosine-1-phosphate, endothelin-1). This phenomenon was also observed on the CellKey. HEK cells transfected with a $G\alpha_i$ coupled receptor show a change in response profile to carbachol (B) but in contrast to the BIND data the response is not abolished in the presence of a over-expressed GPCR indicating that the type of receptor may also play a role in the endogenous responses seen. CHO cells transfected with receptor known to couple through $G\alpha_s$ show greatly reduced response to PGE2 in the transfected system (C).

Figure 2. Choice of cell line is critical when generating over-expression tools
A receptor known to couple through both $G\alpha_i$ and $G\alpha_q$ gave slightly different impedance profiles when stably expressed in two different cell lines. In SHSY5Y neuroblastoma cells (A), the profile is consistent with a $G\alpha_q$ response and cannot be modulated with pertussis toxin. However, when co-expressed with $G\alpha_{16}$ in CHO cells (B), the profile more closely resembles $G\alpha_i$ coupling and is pertussis toxin sensitive.



The effect of surface coating on impedance

- surface coatings are regularly used in cell based assays, often without consideration for effects outside of physical attachment
- in our example poly-D-Lysine (PDL) coating reverses the direction of the CellKey impedance signal from an over-expressed Gαs receptor (Figure 3) but not from an endogenous $G\alpha_s$ response in the same cell line (Figure 5C). This highlights the importance of pathway confirmation with modulators and the impact that over-expression can have on the cellular response profile

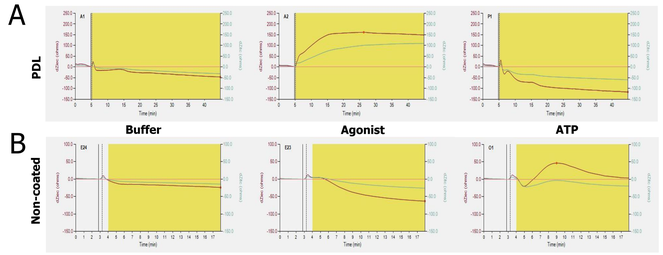


Figure 3. PDL coating changes the CellKey impedance profile of an over-expressed receptor
CHO cells stably expressing a $G\alpha_s$ coupled receptor were treated with an agonist and ATP in the presence (A) and absence (B) of PDL coating. The response profiles are "reversed" where PDL coating is used. However in the same recombinant system the endogenous response to PGE2 is not affected in the same way i.e. not "reversed" by the PDL (data not shown).

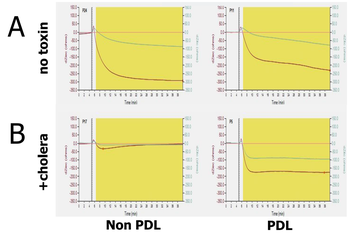


Figure 4. Differential modulation of the endogenous PGE2 signaling by cholera toxin in CHO cells grown on PDL treated plates
Wild type CHO cells also gave a similar response profile to PGE2 on both non-treated and PDL treated plates. However, this response could be abrogated by cholera toxin only on the non-treated surface, suggesting the growth surface has a significant effect on the cellular response.

Complex coupling events on the CellKey

- complex coupling of GPCRs can be detected in CellKey assays (not tested on SRU's BIND platform)
- in our examples, a receptor appeared to have a mixed coupling event that was time dependent (Figure 5). Another receptor known to mix couple through $G\alpha_i/\alpha_q$ (also utilized in Figure 2) displayed concentration dependent mixed coupling (Figure 6)

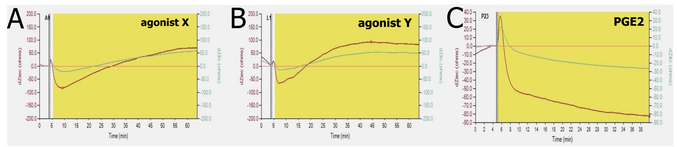


Figure 5. Possible temporally displaced coupling on CellKey
CHO cells transfected with a $G\alpha_s$ coupled GPCR were treated with reference agonist and monitored for 1 hour. The resulting response could be divided into two phases: the initial phase is indicative of $G\alpha_s$ coupling, followed by a second phase (impedance greater than zero) suggesting $G\alpha_i$ signalling. This was observed for two separate reference agonists (A & B) but not in the control $G\alpha_s$ response to PGE2 (C). To be conclusive, modulation with pertussis and cholera toxin would be required.

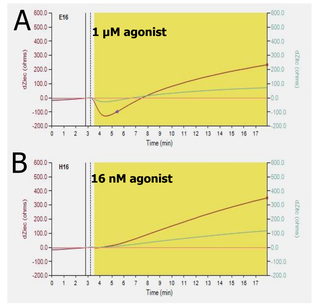


Figure 6. Concentration dependent mixed coupling on CellKey
SHSY5Y cells stably expressing a receptor that is reported to mix couple through $G\alpha_i$ and $G\alpha_q$ were treated with different concentrations of agonist. At high concentrations of peptide ligand a response profile indicative of $G\alpha_q$ coupling was observed (A) whilst at lower concentrations a response profile more indicative of a $G\alpha_i$ signaling was obtained (B). This is in line with published reports but requires modulation to be conclusive.

Data handling: selecting an appropriate metric

- some small molecule screening hits present a challenge with data analysis. Use of a single metric such as maximum response, maximum signal window, or changing the time point at which data are taken, may neglect important subtleties within the kinetic profile. Mixed coupling events will need a more tailored approach to recognise the shift in signaling between the various pathways involved
- the use of label free technologies as an orthogonal platform during a hit identification campaign has the potential to identify responses not apparent in the original screen. Depending on the data export and analysis method, important information regarding the signaling pathways activated and any off target effects may be missed

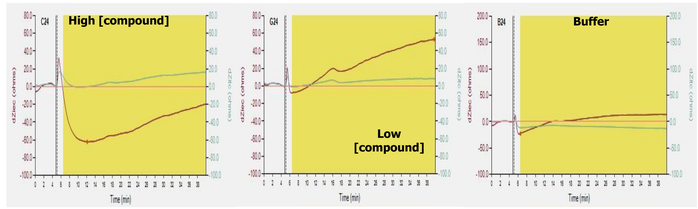


Figure 7. Subtleties in the kinetic profile challenge the data analysis
The CellKey's ability to distinguish between $G\alpha_s$ and $G\alpha_i$ signaling by a positive or negative deviation in the measured impedance requires any data output to reflect the directional nature of the response. The kinetic and, in the case of $G\alpha_q$ signaling, transient nature of the impedance change adds further complexity to the data analysis and careful consideration should be given when selecting an appropriate metric.

Conclusions

Taken together these observations highlight the importance of profiling the model cell system. Particular attention must be paid to the host cell for an over-expression system to ensure that the receptor of interest is giving the desired and expected response. This will allow a better understanding of the cellular response and hopefully the selection of more relevant hit compounds.
Full understanding of the cellular response profiles remains restricted by limited availability of robust and specific modulators of the signaling pathways.
Good experimental design and the need for careful consideration of the data is required to fully utilize and interpret data from these platforms and to permit the full use of these technologies to be realised.

Acknowledgements

The authors wish to thank Cerys Huggins, Simon Lydford, Ryan McGuiness and Jayne Ingram of Molecular Devices for their support and assistance in the generation of the CellKey data.