The use of Dynamic Mass Redistribution (DMR) in the European Lead Factory (ELF): a case study

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The European Lead Factory

The ELF is a public-private partnership aiming to identify hit compounds against molecular targets submitted by European academics and SMEs. These are screened against a Joint European Compound Library (JECL), comprising ~300,000 compounds from seven pharma companies and up to 180,000 compounds synthesised for the project. A key deliverable is a list of up to 50 hit compounds known as the qualified hit list (QHL).

Introduction and assay technology

The ELF portfolio comprises many target classes. This case study describes a Gi/Gq linked GPCR, indicated in neuropathic pain. A cell line stably expressing the target was used to screen ~400,000 compounds in an intracellular Ca^{2+} FLIPR assay producing a provisional hit list of 87 compounds. To provide strong corroborative orthogonal evidence of target engagement and select the QHL, a Dynamic Mass Redistribution (DMR) assay (figure 1) was developed and validated using a series of pharmacological agents.

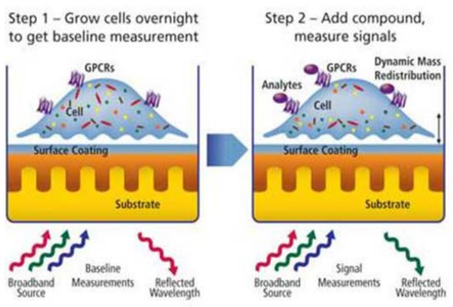

Figure 1: DMR detection. Induction of cellular signalling pathways leads to changes in cellular components (mass) in close proximity to the sensor surface, altering the wavelength of the reflected light (Perkin Elmer, 2013).

Results

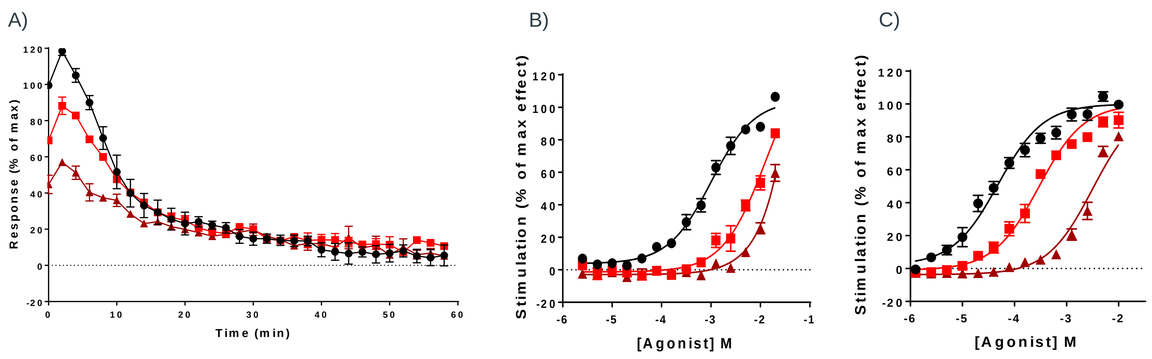

Figure 2: **Three agonists produce a positive DMR response** A) DMR traces for 20mM agonist (B) Normalised dose responses at the peak DMR response at 4 minutes C) Dose responses in FLIPR assay. Agonist 1 (●), Agonist 2 (■), Agonist 3 (▲)

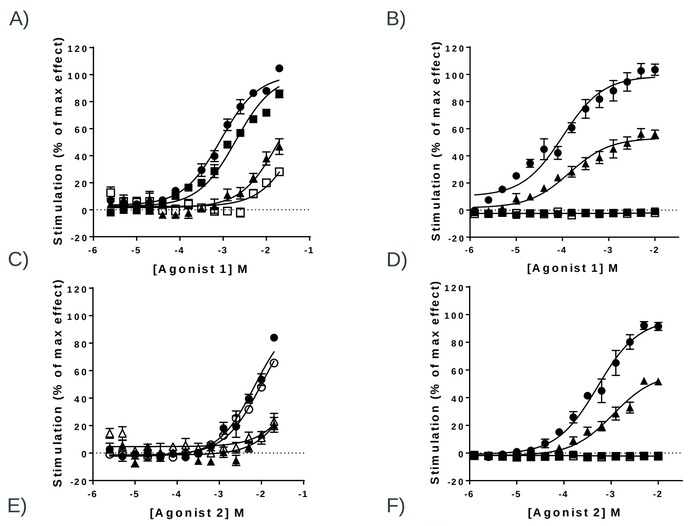

Figure 3: **Agonist effects are coupled through different G proteins in different assay technologies.** Agonist 1 dose responses ± pertussis toxin (PTX) and/or YM-254890 in A) DMR and B) FLIPR. Agonist 2 dose responses ± PTX and/or YM-254890 in C) DMR and D) FLIPR. Agonist 3 dose responses ± PTX and/or YM-254890 in E) DMR and F) FLIPR. Vehicle (●), YM-254890 (■), PTX (▲) and YM-254890 + PTX (□)

Results

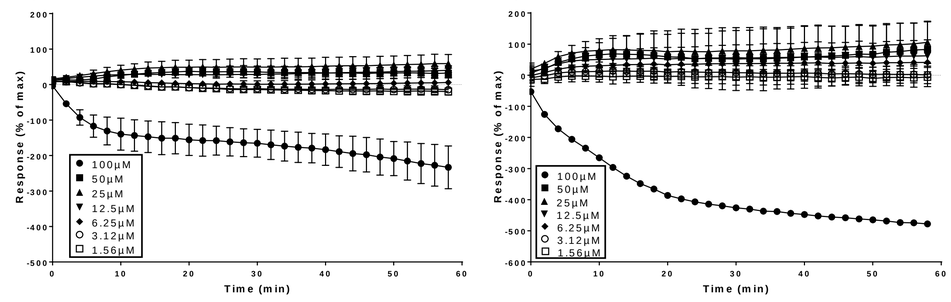

Figure 4: **Antagonist A produces similar DMR effects in A. transfected and B. untransfected cell lines**

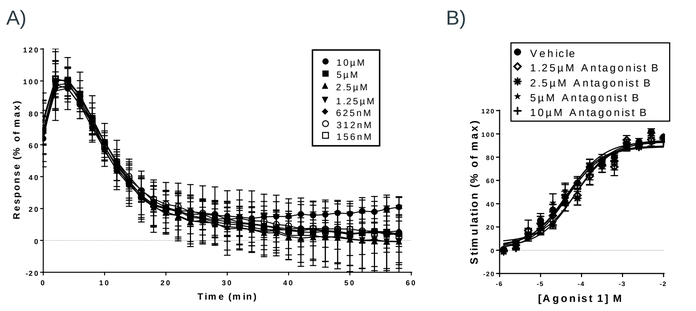

Figure 5: **Antagonist B has no effect on any of the agonists in either DMR or FLIPR** A) DMR response of antagonist B in the presence of 13mM agonist 1 B) Dose response of agonist 1 ± increasing concentrations of antagonist B in FLIPR

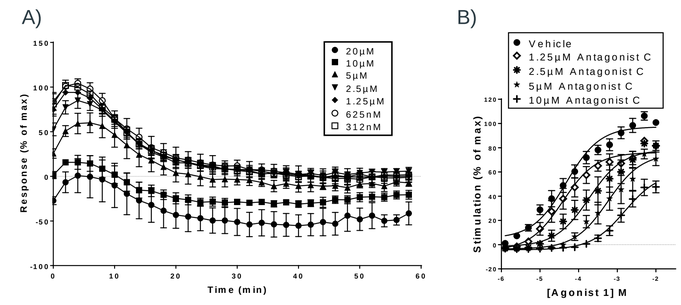

Figure 6: Antagonist C shows a dose dependent antagonism of all agonists in both DMR and FLIPR assays. A) DMR response of antagonist C in the presence of 13mM agonist 1 B) Dose response of agonist 1 ± antagonist C

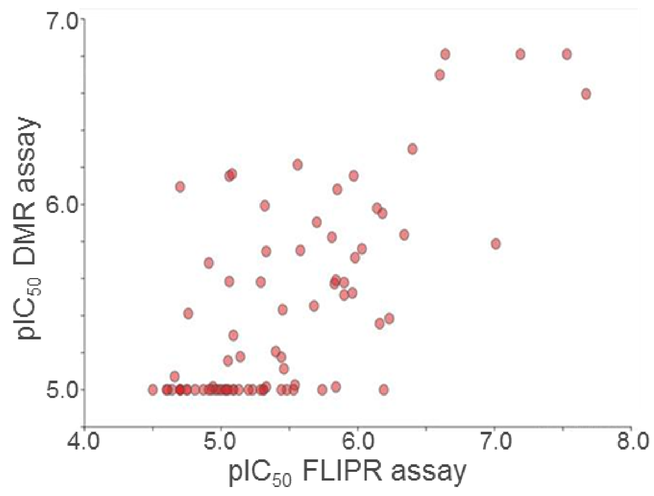

Figure 7: Hit compounds from the ELF screen of ~400,000 compounds show similar potency in the FLIPR and DMR assays The potency of the 87 hit compounds observed in the Ca^{2+} release FLIPR assay (pIC_{50}, x-axis) correlates with that observed in the DMR assay (pEC_{50} y-axis)

Conclusions

- **A DMR assay was successfully developed for an ELF GPCR target**
- **The assay was validated with known agonists which produced a transient response in the transfected but not the untransfected cell lines**
- **These responses were inhibited by a literature antagonist (C)**
- **Two other compounds described in the literature as antagonists (A and B) showed no specific activity for the receptor.**
- **The DMR agonist response was greatly reduced with the Gi/o inhibitor, PTX, and further reduced by the Gq inhibitor, YM254890.**
- **In contrast the FLIPR agonist response was completely abolished by YM-254890 but PTX reduced it by ~50%**
- **Many of 87 screening hits confirmed activity in the DMR assay with good correlation with the FLIPR assay**

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