Integrating Signaling Kinetics into GPCR Compound Profiling

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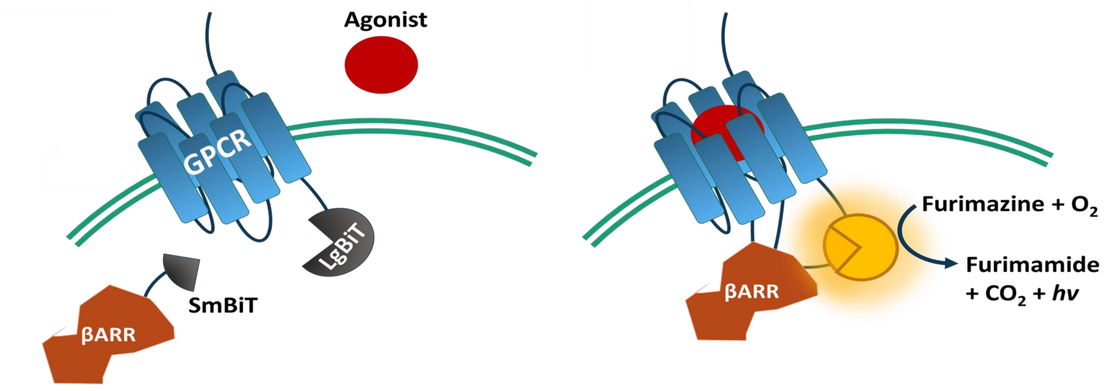
- Optimisation of ligand binding and signalling kinetics is useful to consider in early stage compound profiling
- For G protein-coupled receptors (GPCRs) there is a relationship between the kinetics of signalling pathway activation and desired functional phenotype
- Common GPCR assay are endpoint based with responses measured at different timepoint
- Can new technologies be applied to develop robust kinetic signalling assay suitable for compound profiling?

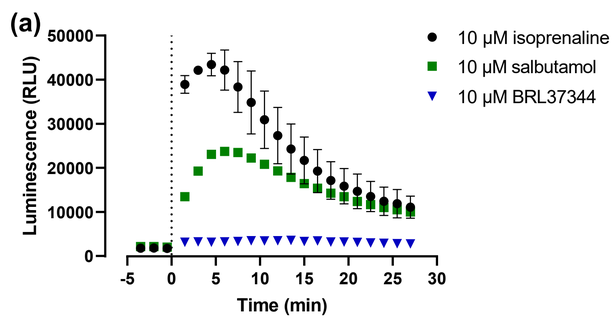
Excellerate Bioscience

- Excellerate Bioscience is a contract research organisation (CRO) specialising in molecular & cellular pharmacology and immunology.
- Based in BioCity Nottingham, our team of drug discovery scientists work on projects with leading pharmaceutical biotechnology & academic institutions around the globe.
- We combines the best of academic and industrial science to develop innovative approaches that will improve the efficiency and translatability of *in vitro* pharmacological profiling.
- Investing in capabilities, R&D methodology and assay platform development

NanoBiT® technology

Promega





- Luciferase complementation assay
- Luminescence measured in real time, in live cells at 37°C
- Cells stably expressing β_2AR-LgBiT and βarrestin2-SmBiT

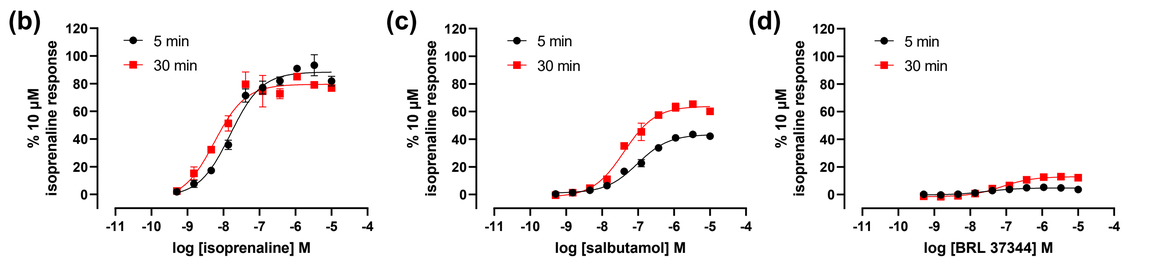


Figure 1 Potency and efficacy of reference βAR agonists in βarresin2 recruitment assay

(a) Kinetic profile of agonist mediated βarresin2-SmBiT recruitment to β_2AR-LgBiT resulting in an increase in luminescence. Concentration response curves for (b) isoprenaline, (c) salbutamol or (d) BRL 37344 at 5 min (black circles) or 30 min (red squares). Concentration response curves can be plotted from any time point in the kinetic read. (e) Average potency values from 4 independent βarresin2 recruitment assays.

(e)

	5 min		30 min	
	pEC_{50}	Rmax (% 10μM iso)	pEC_{50}	Rmax (% 10μM iso)
Isoprenaline	7.29 ± 0.25	100	7.77 ± 0.35	100
Salbutamol	6.50 ± 0.23	29.8 ± 10.4	6.89 ± 0.33	59.8 ± 4.9
BRL 37344	7.63 ± 0.17	4.53 ± 0.42	7.37 ± 0.20	14.2 ± 1.1

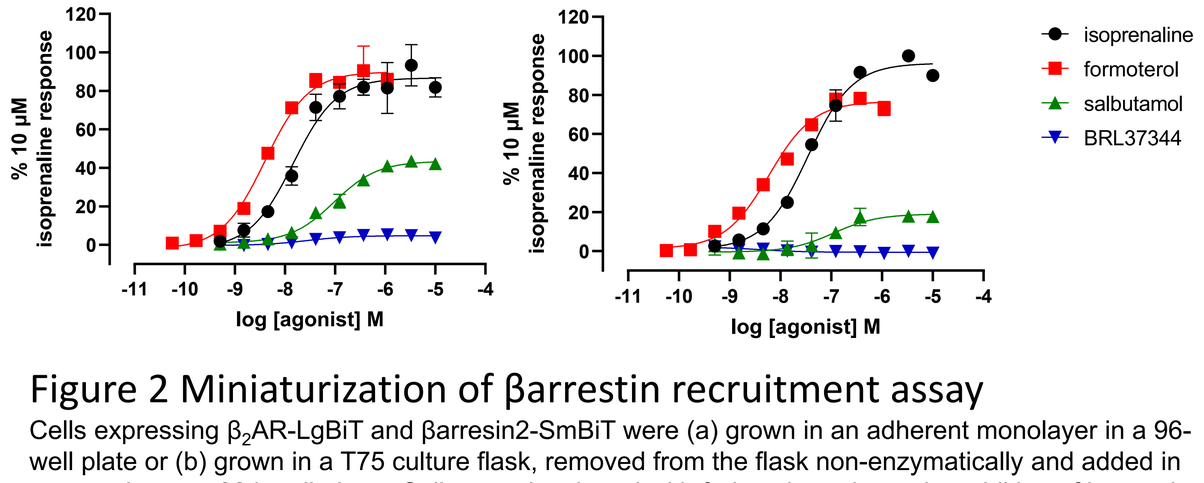


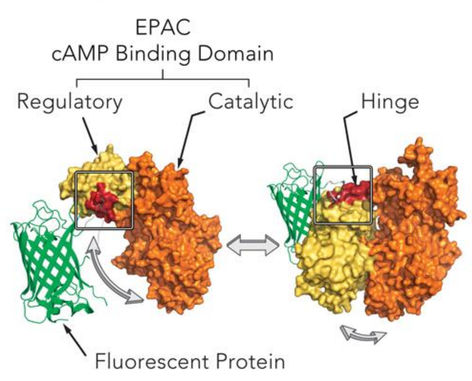
Figure 2 Miniaturization of βarrestin recruitment assay

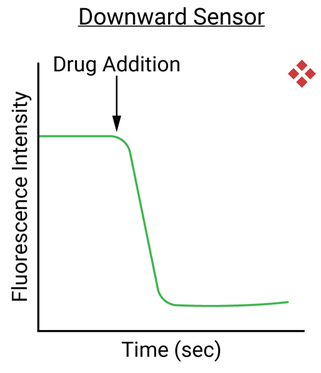
Cells expressing β_2AR-LgBiT and βarresin2-SmBiT were (a) grown in an adherent monolayer in a 96-well plate or (b) grown in a T75 culture flask, removed from the flask non-enzymatically and added in suspension to a 384-well plate. Cells were incubated with furimazine prior to the addition of increasing concentrations of 4 reference agonists. Data shown is 5 min after the addition of agonist and is representative of one experiment performed in duplicate.

cADDis biosensor technology

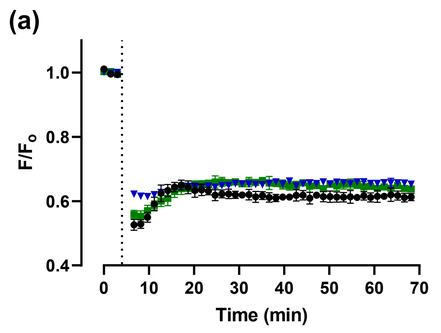
Montana Molecular

Image taken from Tewson et al J Biomol Screen 2016





- Circularly permuted GFP positioned between the catalytic and enzymatic domains of EPAC



- Fluorescence measured in real time, in live cells at 37°C
- cADDis sensor contained in BacMam vector and transduced into cells stably over- expressing β_2AR

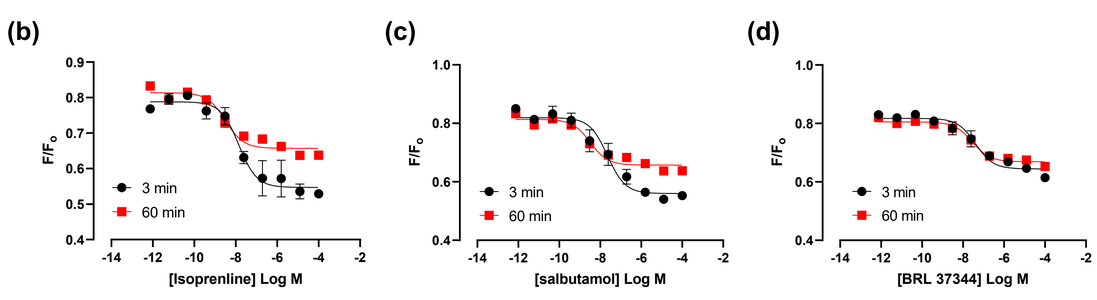


Figure 3 Potency and efficacy of reference βAR agonists in βarresin2 recruitment assay

(a) Kinetic profile of cAMP levels in cells expressing SNAP-β_2 and cADDis downwards sensor upon β_2 stimulation with agonist. Concentration response curves for (b) isoprenaline, (c) salbutamol or (d) BRL 37344 at 3 min (black circles) or 30 min (red squares). Concentration response curves can be plotted from any time point in the kinetic read. (e) Average potency values from 2 independent experiments.

	5 min	30 min
	pEC_{50}	pEC_{50}
Isoprenaline	8.03 ± 0.20	9.18 ± 0.44
Salbutamol	7.52 ± 0.11	8.49 ± 0.02
BRL 37344	6.61 ± 0.59	7.63 ± 0.04

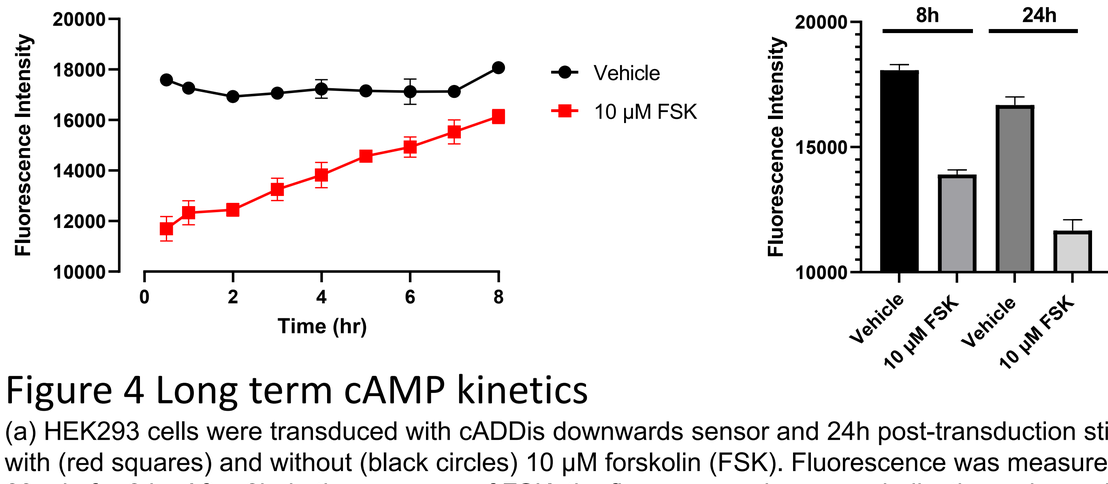


Figure 4 Long term cAMP kinetics

(a) HEK293 cells were transduced with cADDis downwards sensor and 24h post-transduction stimulated with (red squares) and without (black circles) 10 μM forskolin (FSK). Fluorescence was measured every 60 min for 8 h. After 8h, in the presence of FSK, the fluorescence increases indicating a desensitization of FSK-stimulated cAMP production. (b) To confirm responsiveness of the cells to FSK, cells were treated with 10 μM FSK after 8h or 24h in assay buffer.

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- **Promega's NanoBiT to measure βarrestin recruitment and Montana Molecular's cADDis sensor to measure changes in cAMP concentration.**
- **Both these technologies allow the kinetics of agonist responses at β adrenergic receptors to be studied and uncover differences in agonist potency and efficacy profiles.**
- **These technologies provide an ideal opportunity to place the study and optimisation of signalling kinetics at an early stage in lead optimisation.**