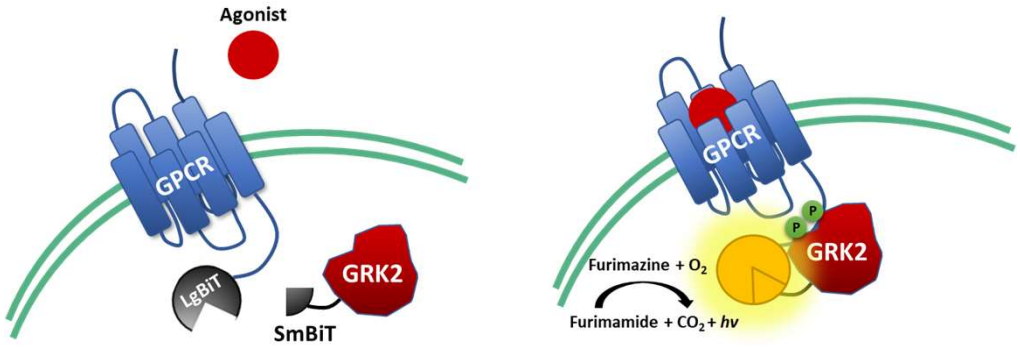
A Luciferase Complementation Approach to Measure GRK2 Recruitment to GPCRs

Mohammed Azim Miah, Julija Sirina, Nicholas D Holliday, Leigh A Stoddart

Excellerate Bioscience, Biocity, Nottingham, UK

- Every step of G protein-coupled receptor (GPCR) activation and regulation pathways displays a unique kinetic profile.
- Agonist bound confirmations of GPCRs are phosphorylated by G protein receptor kinases (GRKs) to desensitize downstream signalling pathways and to prime the receptor for internalization
- There remains few methods to study the kinetics of GRK recruitment to GPCRs. Can we develop a complementation-based approach to measure GRK2 recruitment to GPCRs?

- Excellerate Bioscience is a contract research organisation (CRO) specializing in *in vitro* molecular & cellular pharmacology.
- Our team of drug discovery scientists collaborate with leading pharma, SME & academic institutions around the globe.
- The best of academic and industrial science is combined to develop innovative approaches that will improve efficiency and translatability of *in vitro* pharmacological profiling.
- Target validation, lead optimisation and mechanism of action studies across receptor, enzyme and other target classes, in a full range of disease areas including metabolic disease, immunology and oncology.

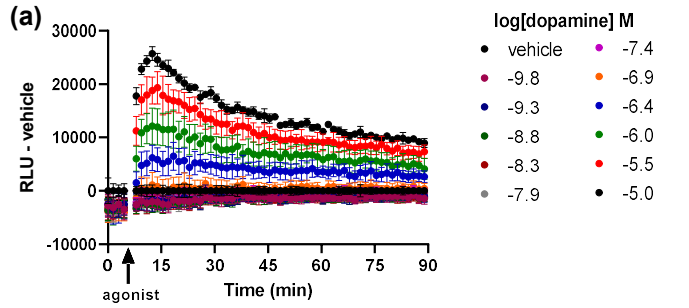


NanoBiT® technology

Promega

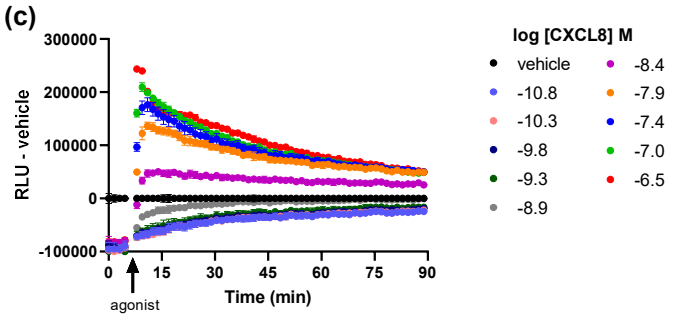
Figure 1. NanoBiT luminescence complementation assay measuring GRK2 recruitment at GPCRs.
The receptor of interest is C-terminally tagged with the LgBiT fragment and the GRK2 is tagged with the SmBiT at the C-terminus. The receptor recruits GRK2 upon agonist stimulation which results in enzyme complementation, and the production of luminescence on addition of the NanoLuc substrate, furimazine.

D_2R-LgBiT + GRK2-SmBiT



	D_2-LgBiT + GRK2-SmBiT	
Time (min)	pEC_{50}	E_{max} (vs 10 min response)
10	5.96	100
20	6.00	74.8
40	6.00	57.3
55	6.08	52.0
85	6.06	36.4

CXCR2-LgBiT + GRK2-SmBiT



	CXCR2-LgBiT + GRK2-SmBiT	
Time (min)	pEC_{50}	E_{max} (vs 10 min response)
10	5.96	100
20	6.00	82.2
40	6.00	63.7
55	6.08	48.2
85	6.06	30.1

Figure 2. Time and concentration dependence of GRK2 recruitment to two prototypical GPCRs
HEK293T cells transiently expressing (a and b) the dopamine D2 receptor-LgBiT (D_2-LgBiT) and GRK2-SmBiT or (c and d) the chemokine CXC receptor 2-LgBiT (CXCR2-LgBiT) and GRK2-SmBiT were grown in an adherent monolayer in a 96-well plate. Cells were incubated with furimazine prior to the addition of increasing concentrations of reference agonists. Data shown is representative of one experiment performed in duplicate.

UKRI Innovate UK

eurostars™

This work was supported in part the Synstar Consortium E114421 which is funded by Innovate UK and Eurostars.

- Using two model GPCRs, the dopamine D_2 receptor and chemokine CXCR2 receptor, we demonstrate that the concentration dependent recruitment of GRK2 can be measured using NanoBiT technology.
- The addition of GRK2 to the toolbox of kinetic profiles that can be measured along the GPCR activation and regulation pathways allow for agonists with different profiles to be selected at the early stages of drug discovery.