Title: Luciferase Complementation Approaches to Measure GRK2 Recruitment to GPCRs

Authors: M. Azim Miah, Julija Sirina, Nicholas D Holliday, Leigh A Stoddart

Affiliations: Excellerate Bioscience LTD, Biocity, Nottingham, UK.

Abstract: Agonist stimulation of G protein-coupled receptors (GPCRs) activate several downstream second messenger signalling pathways. Phosphorylation of agonist bound GPCRs results in receptor desensitisation and trafficking from the plasma membrane consequently inactivating the signalling pathways. Phosphorylation of agonist-bound GPCRs are carried out by G protein receptor kinases (GRKs) of which five act on non-visual receptors. Each step of the GPCR activation and regulation pathways exhibit a unique kinetic profile. Biased agonists, display promising therapeutic approaches to target GPCRs and have been shown to activate different kinetic profiles. Various technologies have been applied to study the kinetics of G protein activation, β-arrestin recruitment, receptor internalisation and second messenger production, but there remains few methods to study the kinetics of GRK recruitment to GPCRs. Here we report the use of Promega's NanoBiT® technology to measure GRK2 recruitment to two agonist stimulated GPCRs. NanoBiT is a complementation approach and is based on the splitting of the luciferase NanoLuc into a LgBiT fragment (157 amino acid) and a SmBiT (11 amino acid) peptide which individually are non-luminescent. However, in close proximity both fragments reconstitute to produce luciferase luminescence. In our hands the GPCR and GRK2 were tagged with LgBiT and SmBiT respectively. Using two model GPCRs, the dopamine D2 receptor and chemokine CXCR2 receptor, we demonstrate that GRK2 can be concentration dependently recruited to the receptor. Using the kinetic profile, concentration response curves can be plotted at different time points, and these can be compared to profiles obtained when examining β-arrestin-2 recruitment. 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