Title: Integrating signaling kinetics into GPCR compound profiling.

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Abstract

In vitro early stage compound profiling often focusses on single timepoint measurements of drug action, for example the affinity of the drug for its target at equilibrium, or the endpoint response in a signalling assay. However *in vivo,* drug action occurs not at equilibrium, but within a highly dynamic system. The concentration of the drug in the vicinity of the target continually changes based on its pharmacokinetic properties, it may be in competition for the target with fluctuating levels of hormones, neurotransmitters or other messengers, and the therapeutic effect itself may depend on a particular pattern of downstream cellular signalling over time.

G protein-coupled receptors (GPCRs) remain a major class of drug target. Agonist molecules that act on at GPCRs stimulate a number of different second messenger signalling pathways that each display a unique kinetic profile. For many second messenger assays, measuring a kinetic profile requires each time point to measured independently. A number of new technologies allow a number of the most common GPCR second messenger pathways to be measured kinetically.

Here we report the use of two of these technologies; 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.