bioSensAll®

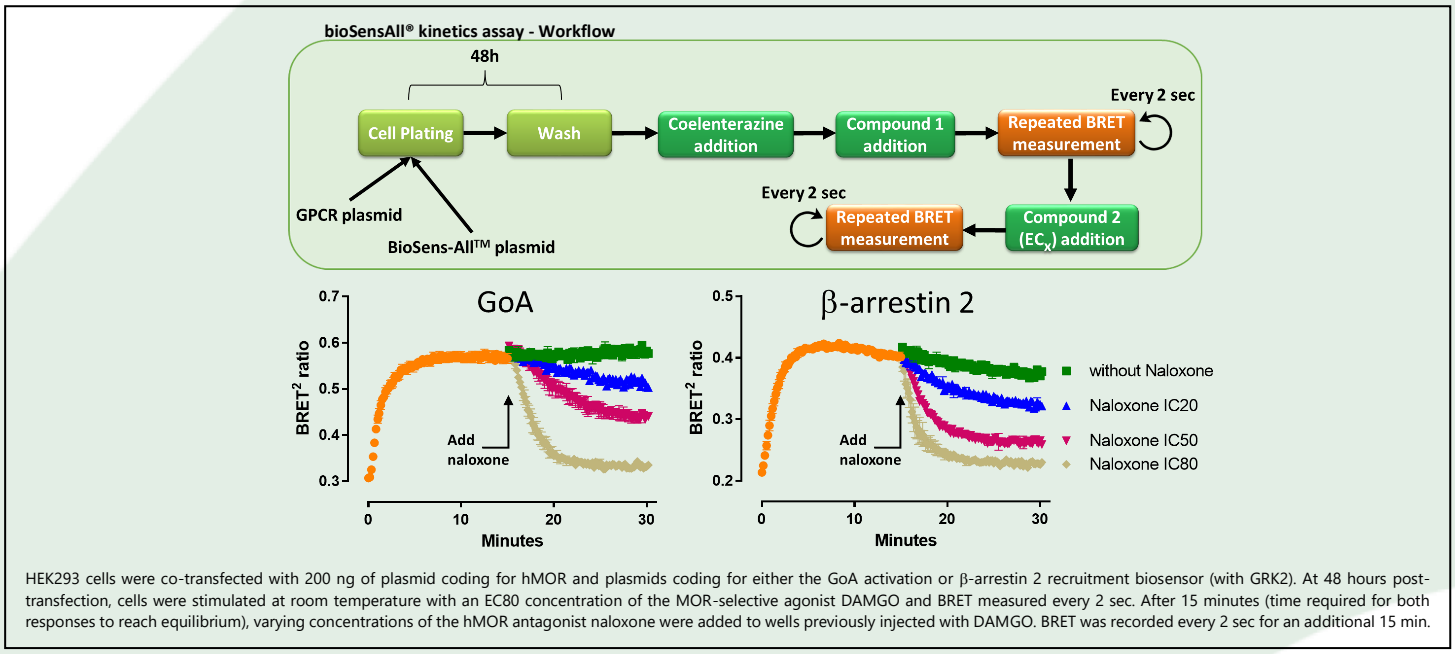
Case Study

Measurement of pathway-specific signaling kinetics in real-time with bioSensAll®

Comprehension of the temporal aspects (i.e., kinetics and dynamics) of receptor-mediated signaling is integral to fully appreciating the mechanisms by which extracellular signals impact cellular behavior. For ligand-receptor interactions, kinetics can be generally described at two levels: i) ligand binding to the target receptor, and ii) receptor downstream signaling. Ligand binding kinetics are defined in terms of k_{on} and k_{off} values (ligand association and dissociation rates, respectively) determined via radioligand binding assays. Signaling kinetics are defined in temporal terms and may range from fractions of a second to many hours.

The effect of kinetics on a ligand's efficacy and biological action(s) has long been recognized but all too often overlooked [1]. Consideration of kinetics is all the more important when dealing with G-protein coupled receptors (GPCRs) given their well-appreciated capacity to activate multiple signaling pathways exhibiting distinct kinetic properties. Further, it is now evident that different ligands of a given GPCR can activate distinct subsets of receptor-coupled signaling cascades, thus leading to compound-specific signaling signatures (a.k.a., biased agonism). Recent observations have suggested that both a ligand's signaling signature and biased qualities may be qualitatively and quantitatively modulated over time [2]. Single point readouts of signaling conducted at equilibrium may therefore provide an incomplete portrait of a ligand's character and fail to capture important differentiating elements between compounds. As a result, the ability to measure signaling kinetics in real-time over short (milliseconds) and extended (hours) timescales offers invaluable insight into a compound's pharmacological properties.

In this application note, we demonstrate how a single, multiple-compound addition assay with **bioSensAll®** biosensors was used to obtain real-time kinetic measurements of G-protein (GoA) activation and β-arrestin 2 membrane recruitment for the human mu-type opioid receptor (hMOR) agonist and antagonist DAMGO and naloxone, respectively.



HEK293 cells were co-transfected with 200 ng of plasmid coding for hMOR and plasmids coding for either the GoA activation or β-arrestin 2 recruitment biosensor (with GRK2). At 48 hours post-transfection, cells were stimulated at room temperature with an EC80 concentration of the MOR-selective agonist DAMGO and BRET measured every 2 sec. After 15 minutes (time required for both responses to reach equilibrium), varying concentrations of the hMOR antagonist naloxone were added to wells previously injected with DAMGO. BRET was recorded every 2 sec for an additional 15 min.

Results and Conclusion – DAMGO-induced GoA activation and plasma membrane recruitment of β-arrestin 2 displayed comparable kinetics post-agonist addition. However, whereas maximal GoA activation was sustained for at least 30 min, the presence of β-arrestin 2 at the plasma membrane declined in a time-dependent manner. Pathway-specific differences in sensitivity to the antagonist activity of naloxone were also revealed. Indeed, naloxone displayed faster kinetics in antagonizing DAMGO-dependent β-arrestin 2 membrane localization (especially visible with naloxone IC50). **bioSensAll®** technology allows for the real-time assessment of ligand signaling kinetics and dynamics across multiple GPCR-downstream signaling pathways and on timescales ranging from milliseconds to hours in a simple and homogenous assay format. bioSensAll® thus provides additional information about ligand pharmacology that cannot be obtained via equilibrium or endpoint assays.

References

[1] Mackay, D. (1977) A critical survey of receptor theories of drug action. *Kinet. Drug Action.* 47:225–321. [2] Klein Herenbrink, C., Sykes, D.A., Donthamsetti, P. *et al*. (2016) The role of kinetic context in apparent biased agonism at GPCRs. *Nat. Commun.* 7. Article number: 10844.

more information: collabdtna@domaintherapeutics.com

more on our website: domaintherapeutics.ca

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