Utilising novel BRET approaches to decipher the complexities of cellular signaling

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Profiling in live cells and in real time can provide valuable insights into GPCR pharmacology, including ligand binding, G protein coupling, arrestin recruitment, internalization, trafficking and recycling, as well as interactions with a variety of other regulatory and signalling molecules. Furthermore, the complexity of GPCR signalling systems is magnified by the concepts of biased signalling, allosterism and receptor oligomerisation. Bioluminescence resonance energy transfer (BRET) is a powerful technique that can be used to investigate all these aspects of GPCR pharmacology, through real-time monitoring of molecular proximity in live cells.

Over the last decade, alongside technological improvements, development of these approaches has resulted in improved assays as well as novel tools to explore further aspects of GPCR function in greater detail. We have been at the forefront of developing these approaches and recent highlights from our laboratory will be discussed. These include (1) developing the NanoBRET ligand binding assay enabling real-time, live cell monitoring of ligand-receptor interactions for both small molecules and peptides [1]; (2) further developing the BRET trafficking assay pioneered by Nevin Lambert to enable real-time, live cell monitoring of protein trafficking from the plasma membrane to various subcellular compartments [2]; and (3) to overcome the fundamental limitation of BRET assays requiring ectopic over-expression, our most recent developments showing the first demonstration of BRET using CRISPR/Cas9 genome editing technology to incorporate BRET-tags onto proteins under endogenous promotion to monitor receptor function [3].

These novel approaches are powerful and complement investigations using more established receptor-G protein and receptor-arrestin BRET proximity assays. The use of such techniques to establish comprehensive kinetic profiling of receptor complexes enables improved understanding of signaling biases with and without the added complexity of heteromerization. Further coupling of BRET approaches with cutting edge gene-editing techniques will allow greater insight when deciphering the complexities of cellular signaling.

1. Stoddart LA et al. (2015). *Nature Methods* 12: 661-663
2. Tiulpakov A et al. (2016). *Molecular Endocrinology* 30: 889-904
3. White CW et al. (2017). *Scientific Reports* 7: 3187