Using BRET Ligand Binding in the Detection and Characterisation of Receptor Heteromers

G protein-coupled receptor (GPCR) heteromerisation results in enhanced complexity within GPCR signalling systems, broadening the scope for drug discovery with the potential for finding improved drug targets. Due to its ability to detect protein-protein interactions in real-time, bioluminescence resonance energy transfer (BRET) has become one of the predominant tools for studying GPCR pharmacology, including the identification and characterisation of GPCR oligomers. In addition to its application for studying protein-protein interactions, recently BRET has also been used to investigate interactions between GPCRs and their ligands (1). This study has adapted the BRET ligand binding assay to investigate GPCR heteromers.

In the standard BRET ligand binding assay, the receptor is N-terminally tagged with the NanoLuc (Nluc) enzyme, and a selective ligand is tagged with the acceptor fluorophore. Upon binding of the ligand to the receptor, energy is transferred from Nluc to the fluorescent ligand, resulting in an increase in the BRET signal. This is indicative of ligand binding, and the specificity of this signal can be confirmed using a competing ligand. In the adaptation of this assay to investigate receptor heteromers, a version of the Receptor-Heteromer Investigation Technology (2), an untagged receptor is coexpressed with the Nluc-tagged receptor. Using a ligand selective for the untagged receptor, the proximity of the two receptors can be monitored through the generation of a specific BRET signal. This study has tested the heteromer binding assay using the angiotensin II (AngII) type 1 and type 2 (AT_1-AT_2) receptor heteromer. Here, BRET was measured from Nluc-AT_1 to a fluorescent AngII ligand (TAMRA-AngII) bound to the untagged AT_2 receptor. The specificity of this signal was verified, as the AT_1 receptor had been blocked with olmesartan and the signal could be reduced through treatment with an AT_2 selective ligand (PD123319). Furthermore, the $\log IC_{50}$ of the PD123319-induced TAMRA-AngII displacement was not significantly different to the $\log IC_{50}$ of PD 123319-induced TAMRA-AngII displacement at Nluc-AT_2 (-6.5 ± 0.1 vs -6.3 ± 0.1, respectively; $p > 0.05$).

This study has illustrated a new approach for investigating receptor heteromers, and shown how heteromer ligand binding can be investigated with BRET for the first time.

(1) Stoddart, LA et al (2015) *Nat Methods* **12**: 661–663.
(2) Jaeger, WC et al (2014) *Front Endocrinol* **5**: 26.