**A novel GPCR heteromer demonstrates unique pharmacology revealed with live cell biosensors.**

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**Introduction.** Chronic inflammation and organ fibrosis underpins much of the morbidity and mortality of many common diseases, such as chronic obstructive pulmonary disease, stroke, cirrhosis, diabetes and chronic kidney disease. There is clearly an unmet need in controlling the complex pathophysiological inflammatory axes of these diseases effectively. G protein-coupled receptors (GPCRs) are prominent drug targets, due to their cell membrane expression, and ability to modulate physiology. Some GPCRs are shown to form receptor heteromers, complexes composed of two different GPCRs with distinct pharmacology from the component protomers. Given the molecular complexity of these diseases, GPCR heteromer discovery may yield effective targets with profound pharmacology distinct from monomers. This project, linked with an industry partner, aims to discover and profile heteromers with a strong scientific rationale and commercial potential.

**Aims**. To identify novel GPCR heteromers and elucidate their unique molecular pharmacology.

**Methods**. Receptor-Heteromer Investigation Technology (HIT) identifies receptor-receptor proximity. The β-arrestin2 recruitment bioluminescence resonance energy transfer (BRET) Receptor-HIT assay was conducted to screen for proximity of GPCR combinations of interest. Positive ‘hits’ proceeded toward pharmacological characterisation with BRET sensors for intracellular trafficking, G protein activation and second messenger generation.

**Results.** Receptor-HIT identified multiple novel heteromer candidates, with a selected candidate proceeded toward comprehensive pharmacological profiling. The intracellular trafficking sensor revealed an asymmetrical perturbance to the internalisation of protomers upon their coactivation. The G protein and second messenger sensors revealed complementary effects upon protomer coactivation, observing an asymmetrical loss of agonist potency. A selective antagonist for one protomer rescued the loss of potency induced by protomer coactivation.

**Discussion.** We have identified a novel candidate, Het-3X, demonstrating pharmacology that satisfies the criteria in classifying a putative GPCR heteromer. This candidate will undergo further scientific and commercial validation.