Structure determination of GPCR heteromers

Alok Pradhan[1,2], Laura Humphrys[1,2], Jason Cao[1,2], Matthew Belousoff[1,2], Denise Wootten[1,2], Patrick M Sexton[1,2]

[1]ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3052, Australia

[2]Drug Discovery Biology Theme, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3052, Australia

G protein coupled receptors are known to form homomers and heteromers to form novel receptor complexes that have distinct pharmacology from its individual promoters. The Angiotensin Receptor Type 1 (AT1R) is known to form homodimers and promiscuously form heterodimer with other GPCRs, including the members of the chemokine family, CCR2 and CXCR2. However, the molecular mechanism underlying heteromer formation and the alternation in the pharmacology and signalling of the receptor complexes have not been well understood. We have generated constructs of the AT1R, CCR2 and CXCR2 with different epitope tags and BRET sensors which will be used in performing G-protein recruitment assay, GPCR-HIT assay and also purification of the protein complexes. The constructs generated have been validated using TruPath assay. Optimization for G protein recruitment assay for monomer and heteromers has been done by testing different assay formats in whole cells as well as semi-permeabilized cells. These experiments will guide the receptor, G protein and ligand combination which supports formation of stable complex which will then be used for structure determination using cryo-electron microscopy.