Monitoring the interactions of μ-Opioid receptors and G-proteins with Fluorescence Cross-Correlation Spectroscopy

Pillai, R.[1], Santiago, M.[2], Graus, M.[1], Pandzic, E.[3], Finch, A.[4], Gaus, K.[1] and Sreenivasan, V.[1]

[1] EMBL Australia Node in Single Molecule Science, School of Medical Sciences, UNSW and ARC Centre of Excellence in Advanced Molecular Imaging, UNSW, Australia, [2] Department of Biomedical Sciences, Macquarie University, Australia, [3] Biomedical Imaging Facility, UNSW, Australia, [4] Department of Pharmacology, School of Medical Sciences, UNSW, Australia

Advanced fluorescence-based techniques are increasingly being used to track the dynamics and interactions of individual molecules within cells [1]. Fluorescent (cross)-correlation spectroscopy (F(C)CS) is a versatile technique used to measure the number of molecular species and diffusion coefficients in bulk and on cell membranes [2]. In this work, we investigate the application of FCCS, to elucidate the molecular coupling of the -opioid receptor and its intracellular signaling complex, G-proteins.

The -opioid receptor (MOR) belongs to the large family of G-protein coupled receptors and is the primary target of analgesic opioid drugs prescribed for acute and chronic pain, such as morphine [3, 4]. Intracellular responses to the binding of the opioid ligands are mediated in part by the heterotrimeric inhibitory G-protein complex, in addition to further signalling mediated by kinase cascades and arrestin-association [4]. The exact nature of the coupling between the GPCRs and G-protein is not well understood. Cellular-level studies based on fluorescence resonance energy transfer (FRET) has been inconclusive [5, 6]. A recent study using 2-colour single molecule tracking suggests a collisional coupling model where the on- and off-rates of the coupling between adrenergic receptors and G-proteins are modulated by the ligand binding [7]. The nature of the G-protein coupling in the case of the MOR is not yet known.

We report on the application of an FCCS-based approach to elucidate the coupling of fluorescently-tagged MOR and G-proteins. From the FCCS data, we aim to measure the number densities of the free and bound, MOR and G-proteins. This will allow us to determine the extent of basal coupling of G-proteins to MOR, and how it is modulated by ligands and/or allosteric modulators.

Acknowledgements:

V. S. acknowledges the Australian Research Council for DE160100888.

R. P. acknowledges the contributions from Zhengmin Yang.

References:

1. Tian, H., A. Fürstenberg, and T. Huber, *Labeling and single-molecule methods to monitor G protein-coupled receptor dynamics.* Chem. Rev, 2017. **117**(1): p. 186-245.
2. Jameson, D.M., J.A. Ross, and J.P. Albanesi, *Fluorescence fluctuation spectroscopy: ushering in a new age of enlightenment for cellular dynamics.* Biophysical reviews, 2009. **1**(3): p. 105.

3. Al-Hasani, R. and M.R. Bruchas, *Molecular mechanisms of opioid receptor-dependent signaling and behavior.* Anesthesiology: The Journal of the American Society of Anesthesiologists, 2011. **115**(6): p. 1363-1381.
4. Oldham, W.M. and H.E. Hamm, *Heterotrimeric G protein activation by G-protein-coupled receptors.* Nature reviews Molecular cell biology, 2008. **9**(1): p. 60-71.
5. Hein, P., et al., *Dynamics of receptor/G protein coupling in living cells.* The EMBO journal, 2005. **24**(23): p. 4106-4114.
6. Galés, C., et al., *Real-time monitoring of receptor and G-protein interactions in living cells.* Nature methods, 2005. **2**(3): p. 177-184.
7. Sungkaworn, T., et al., *Single-molecule imaging reveals receptor-G protein interactions at cell surface hot spots.* Nature, 2017.