Imaging Nano-domains in Mammalian Membranes Using Super-Resolution Microscopy and Advanced Analysis

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Background

The angiotensin II type 1 receptor (AT1R) is a membrane receptor protein, which plays a large role in the cardiovascular physiology. The receptor can cause two different pathways within the cell, where one is a G-protein mechanism, while the second is a β-arrestin2-depent pathway. It is shown that the G-protein pathway can have pathogenic effects if the stimulation time of the receptor is too high [1]. Initial indications show that there is a difference in nano-domain formation depending on the pathway.

Motivation

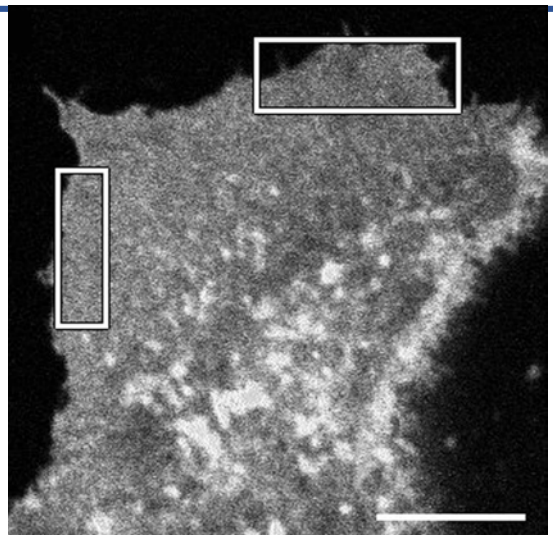
It has recently been accepted that most membrane proteins are present in nano-domains. [2]. Due to it being a relatively new discovery there is not a lot of further knowledge on nano-clusters. Further knowledge of the membrane dynamics will add to this knowledge, which can have great interest to the pharmaceutical industry.

Objectives

The objective of this work is to obtain knowledge of AT1R dynamics in the plasma membrane on live mammalian cells using super-resolution microscopy. Furthermore, development and optimization of new and existing analysis software for imaging data will be made.

Methods

- Growing mammalian cells, which will be transfected with the plasmid expressing the protein of interest.
- Image live mammalian cells using fluorescent markers on the membrane protein of interest using various microscopic techniques (e.g. PALM and TIRF).
- Analyse the images using various analysis methods including STICS [3] and kICS [4].
- Optimize the analysis method currently used to fit with the recent technological development.



Example of a bioimage of a live mammalian cell being fluorescently marked by green fluorescent protein [5].

[1] M. Aplin *et al.*, "Differential extracellular signal-regulated kinases 1 and 2 activation by the angiotensin type 1 receptor supports distinct phenotypes of cardiac myocytes," *Basic Clin. Pharmacol. Toxicol.*, vol. 100, no. 5, pp. 296–301, 2007.
[2] M. F. Garcia-Parajo, A. Cambi, J. A. Torreno-Pina, N. Thompson, and K. Jacobson, "Nanoclustering as a dominant feature of plasma membrane organization," *J. Cell Sci.*, vol. 127, no. 23, pp. 4995–5005, 2014.
[3] B. Hebert, S. Costantino, and P. W. Wiseman, "Spatiotemporal image correlation spectroscopy (STICS) theory, verification, and application to protein velocity mapping in living CHO cells," *Biophys. J.*, vol. 88, no. 5, pp. 3601–3614, 2005.
[4] D. L. Kolin, D. Ronis, and P. W. Wiseman, "k-space image correlation spectroscopy: A method for accurate transport measurements independent of fluorophore photophysics," *Biophys. J.*, vol. 91, no. 8, pp. 3061–3075, 2006.
[5] E. C. Arnspang, J. S. Koffman, S. Marlar, P. W. Wiseman, and L. N. Nejsum, "Easy Measurement of Diffusion Coefficients of EGFP-tagged Plasma Membrane Proteins Using k-Space Image Correlation Spectroscopy," *J. Vis. Exp.*, no. 87, pp. 1–9, 2014.

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