COMPARE

CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

Newsletter

Edition 10, September 2018

Congratulations

Congratulations to **David Hodson,** Professor of Cellular and Metabolism and COMPARE PI, who has been awarded a Fellowship of the Royal College of Veterinary Surgeons (FRCVS). The FRCVS is only awarded to those held in the highest esteem by the profession.

Comment from David "*I'm really pleased to have been awarded FRCVS. I am hoping that this will show veterinarians thinking about careers in basic research that it is a) very rewarding; and b) recognised by the profession. It is my belief that veterinarians can make a great contributions to medicine.*"

Grant Successes

Wellcome Trust Senior Research Fellowship, Davide Calebiro, University of Birmingham, has been awarded a Wellcome Trust Senior Research Fellowship.

Project title; 'Towards a Single-Molecule Pharmacology of G-Protein-Coupled Receptors: Understanding Receptor Dynamics to Develop Innovative Drugs'

Additional COMPARE grant successes include BHF Infrastructure Grant, BBSRC Industry Grant with UCB, CAPES (Brazil) UoN Drug Discovery Programme, GSK Respiratory Phenotypes for genetic evaluation, iCase Studentship (GSK).

Welcome to COMPARE

We are pleased to welcome the following colleagues to COMPARE;

Meri Canals, *Chair and Professor of Cellular Pharmacology* and **Rob Lane,** *Research Fellow and Assistant Professor Molecular Pharmacology will join us on the 1st October 2018.*

Caroline Gorvin, *Research Fellow in Metabolism and Systems Research*. Caroline is using cutting edge imaging and cell biology techniques to study the cell biology of metabolic G protein-coupled receptors.

Dylan Owen, *Interdisciplinary Chair in Immunology and Mathematics*, Dylan's research interests are in the development and application of cutting-edge fluorescence microscopy techniques and their applications to cell biology, in particular the study of cell membranes and T Cells.

Key Dates

Annual Research Symposium
Edgbaston Park Hotel and Conference Centre,
University of Birmingham
28th September 2018

Sandpit Event, Birmingham
"*Investigating receptor dynamics by single-molecule microscopy*" 26th October 2018
13:30-17:30
IBR Seminar Room
University of Birmingham

Welcome PhD Student Event
1st November 2018
University of Birmingham and
13th November 2018
University of Nottingham
Details to follow

Inaugural Lecture
Davide Calebiro
7th November 2018 16:30
University of Birmingham

Sandpit Event, Nottingham
"*Gene editing and stem cell approaches in the study of membrane proteins*"
23rd January 2019 14:00-17:00
C1052 Medical School
University of Nottingham

Workshop *Single-Molecule imaging and spectroscopy of membrane proteins*
12th & 13th March 2019
CPD, University of Birmingham Medical School

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If you have any items for the next newsletter please send to:

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Microscopes—Olympus LV200 Livecyte—a bioluminescence microscope, Joëlle Goulding, UoN

The Olympus LV200 is an inverted microscope setup which allows the imaging of bioluminescence. As there is no need to excite the sample as you would do in fluorescence imaging, bioluminescence offers improved signal-to-noise ratio and no phototoxicity nor photobleaching. The LV200 is encased in a light-tight dark box and there is minimal distance between your sample and the EM-CCD camera allowing maximal light collection and high-quality images. Due to this sensitivity image acquisition is fast allowing for the study of kinetics. The LV200 microscope is ideal for taking single cell bioluminescence readings and due to the environmental chamber samples can be imaged over longer time periods. Our system has the following objectives; 60xOil (1.42NA), 40xOil (1.3NA) 40x (0.6NA), 20x (0.75NA), 10x (0.4NA).

At Nottingham we have been working to develop BRET imaging on the LV200. BRET, Bioluminescence Resonance Energy Transfer, is the transfer of energy between a bioluminescent donor and a fluorescent acceptor in close proximity which lends itself perfectly to the study of receptor-ligand binding. We have combined the nanoluciferase technology as donor emitter with fluorescent ligands as acceptors.

This is exactly what Diana Alcobia, a final year PhD student within COMPARE at Nottingham has been doing. Diana has recently had her first, first-author paper 'Visualising ligand-binding to a GPCR in vivo using nanoBRET' published in iScience (vol 6. 31 August 2018, 280-288 https://doi.org/10.1016/j.isci.2018.08.006) combining work done in Nottingham, under the supervision of Professor Steve Hill, and in Monash Australia, under the supervision of Associate Professor Erica Sloan. Here she is giving her thoughts on the LV200;

"I found the LV200 luminescence imaging system very user friendly. This is an ideal system to complement our NanoBRET-based assays to measure ligand-receptor binding kinetics, as it allows the visualisation where specific ligand-receptor interactions are occurring within living cells. For *instance, β-adrenergic antagonists (clinically known as beta-blockers) have shown therapeutic effect in preventing breast cancer invasion and metastasis. Using the LV200 microscope, we were able to determine the location of specific binding of a fluorescently-labelled β-adrenoceptor antagonist (Propranolol-β-Ala-β-Ala-X-BY630/650) to NanoLuc-tagged β_2-adrenoceptors overexpressed in triple negative breast cancer cells."*

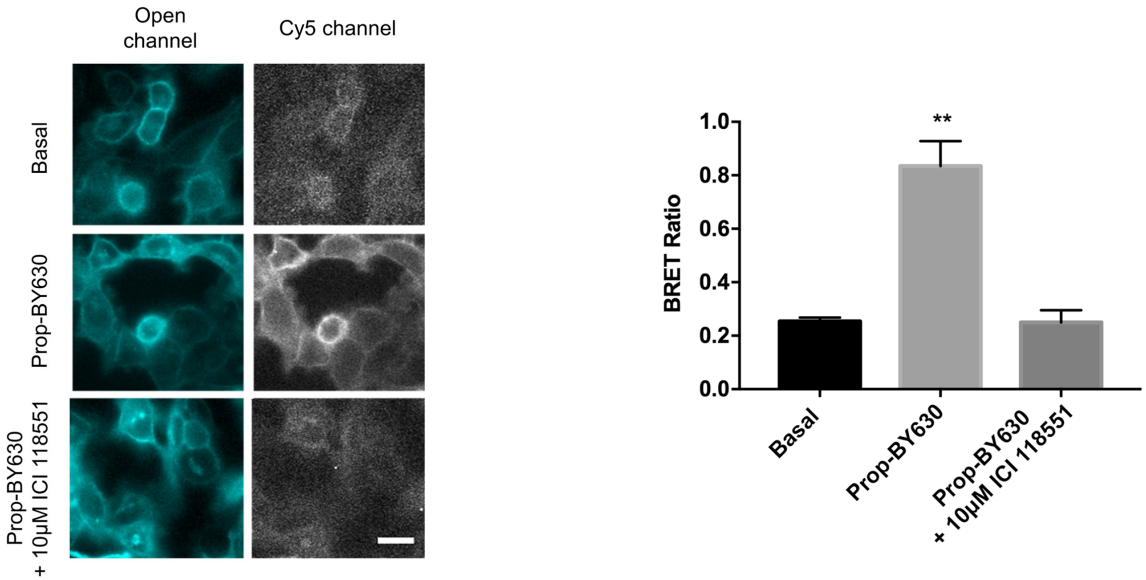

***Figure**: Bioluminescence imaging (Olympus LV200) of NanoLuc-tagged β2-adrenoceptors. MDA-MB231HM Nluc-β2AR cells treated with 400nM furimazine substrate alone (upper panels) to detect luminescence in the absence of added fluorescent ligand using an open channel (20 sec exposure time; 420nm longpass filter; upper left panel) or a CY5 channel (4 min exposure time; 600/50nm bandpass filter; upper right panel; to detect BRET generated by binding of fluorescent ligand, when present). Middle and lower panels show images from cells treated with 50nM Prop-BY630, in the presence (lower panels) or absence (middle panels) of unlabelled ICI 118551 (10μM). Images shown were acquired with an open channel (middle and lower left panels) and the CY5 channel (middle and lower right panels). Scale bars represent 50μm.*

***Graph**: BRET ratios obtained using bioluminescence imaging using ImageJ time-series analyser. Data show the mean and S.E. obtained in 3 independent experiments. ** p<0.01 compared to basal or in the presence of 10μM ICI 118551 (One Way ANOVA with Tukey multiple comparisons).*