COMPARE

CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

Newsletter

Edition 24 March 2020

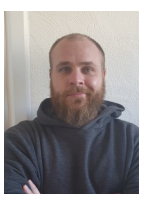
These are unprecedented times and we hope that you and families are well and adjusting to this new way of working. We expect to be in 'lockdown' for several months and it is critical that we stay in touch with our group members through video conferencing and equivalent and that we draw up plans of work for this period. We have set up a meeting between ourselves and the Directors-elect (yet to be formally announced) for just after the Easter period. We will be discussing new initiatives to promote the research in COMPARE during this period. We are holding the date for the COMPARE Symposium in September and are about to send out invitations to speakers acknowledging that we may have to cancel. We wish you all the very best and for keeping positive and virus free.

Good News Stories

Congratulations to **Dirk-Peter Herten** who has been awarded an **Academy of Medical Sciences Professorship**. Dirks' research aims at quantitative microscopy approaches to measure protein concentrations, protein copy numbers and binding affinities by use of novel single-molecule techniques. Current focus is on T cell signalling which plays an important role in innate immune response, e.g. on viral infections and thrombo-inflammation. Using single-photon detection and super-resolution microscopy, he is planning to establish a quantitative 3D reconstruction of cellular structures and whole cells reflecting protein copy numbers that hopefully enable quantitative modelling of the underlying signalling cascade. In the long run, the aim is to generalise the method to make it accessible to other researchers on campus, within COMPARE, and if possible within UK.

Congratulations to **Dr Paul Brady**, Clinical Research Fellow Institute of Cardiovascular Sciences, who has been awarded a grant from the **British Heart Foundation (BHF) Turing Fund**. The project is for 12 months and is "Defining clusters of patients with atrial fibrillation at risk of heart failure and death". As a result of this award, Paul has declined the COMPARE Clinical Fellowship with Drs Steve Thomas and Mark Thomas that he was awarded last year

Congratulations to **Rob Lane** who was awarded the **2019 BPS Novartis Prize**—This prize recognises the achievements in published research of members and is supported by a donation from Novartis. Winners will receive the equivalent of $3,000. Nominations are open for the 2020 award.



Well done to **Rob Hill** who has been award the **2020 ASCEPT-BPS Outstanding Young Investigator Award** – this will see him travel to Australia later in the year to establish collaborations with the Florey Institute in Melbourne.

This award was established by the British Pharmacological Society and the Australasian Society for Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) to provide a significant career-enhancing opportunity by offering a three-week visit to Australia for post-PhD, early-career researchers and supporting participants' accommodation, international and domestic travel, and subsistence during this time (up to £5,000)

Key Dates

COMPARE Annual Research Symposium
24th September 2020
Edgbaston Park Hotel
Birmingham

birmingham-nottingham.ac.uk/compare

Follow us on Twitter
@compare_uobuon
@uobuonpartners

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

compare@birmingham-nottingham.ac.uk

Best wishes in your new position

Paulus Kirchhof is taking on a new role as Director of the Department of Cardiology and Chair in Cardiology at the University Heart Centre Hamburg on 1 April.

Roy Bicknell will take over as the Interim Director of ICVS. **Steve Watson** will take over as the Head of the BHF Accelerator Award. The accelerator award supports research in cardiovascular sciences across the university, including early career researchers and interdisciplinary research.

COMPARE Sandpit—11th March 2020

A COMPARE Sandpit on Machine Learning and Computational Modelling was held in Birmingham on 11 March, with around 40 attendees from both universities. The first session examined the application of machine learning techniques for image analysis, with talks from Jeremy Pike (UoB) on content-aware image restoration, Eva Frickel (UoB) on analysing host response to microbes, and Dylan Owen (UoB) on machine learning for single molecule cluster analysis. A second session saw talks from Mireia Jimenez Roses (UoN) on prediction of activity cliffs, Yann Lanoisellee (UoN) on transient caging, Keverne Louison (UoN) on receptor dimerization, and Anna Simmonds (UoB) on structural analysis of peptides with mass spectrometry and molecular modelling. Excellent discussion followed, with a particular focus on how non-experts could get started in applying these methods to their problems. Many thanks to all the speakers for their excellent talks, and to all who came for a lively and productive discussion.

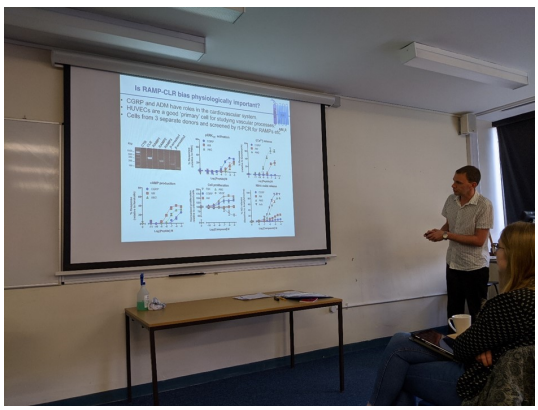
COMPARE Dual PhD Students' Presentation Day—26th February 2020

On the 26th February, the five PhD students directly funded by COMPARE, now in the penultimate year of their programme, presented their results to date and discussed the future directions of their projects with COMPARE colleagues.

Tremendous talks were given by the students, highlighting how much progress they have made during the first half of their PhDs. Audience members at all different stages of their careers, from professors to students, gave terrific questions probing into the student's work and enthralling scientific discussions were had.

To follow the student's presentations, Dr Graham Ladds from the University of Cambridge, who has recently been appointed an associate member of COMPARE, gave an engaging talk about Receptor Activity Modifying Proteins (RAMPs). COMPARE members had plenty of questions for Graham about his work and discussions were continued over refreshments after the talks.

The atmosphere throughout the day was positive, friendly and welcoming. This event highlighted how this centre has grown and how collaborations have blossomed across the two institutions over the last few years. The ICS tearoom was filled with chatter and laughter at the end of the day, a sign of a successful event!



Dr Graham Ladds from the University of Cambridge presenting his research.

COMPARE Dual PhD Student - Project Summary

Evelyn Garlick (Evie)

Talk Title: Using advanced microscopy techniques to investigate the role of actin in the organisation of Adenosine receptors

Investigating the organisation of GPCRs in the cell membrane can help us to understand how signalling can be modulated. There is significant evidence that the cortical actin cytoskeleton can mediate the organisation of some receptors including GPCRs, either through direct interaction or the trapping of receptors in fenced regions of membrane known as corrals. My project aims to investigate the potential interaction of actin and the adenosine receptors A2A and A2B. These receptors are of increasing clinical interest, but little is currently known about their organisation.

To investigate the role of actin in adenosine receptor organisation, we have applied a range of super resolution and advanced microscopy techniques. Stimulated and unstimulated receptor clustering with and without the presence of the actin inhibiting drug cytochalasin D was investigated using dSTORM single molecule microscopy. This revealed that A2B cluster formation and maintenance appears to be unaffected by actin inhibition, while A2A clusters become significantly smaller. This is ablated in stimulated receptors. In terms of dynamics, single particle tracking and subsequent analysis revealed a drop in the immobile fraction of A2B receptors when treating with cytoD. We are currently working on incorporating live actin imaging into this technique, using SRRF image analysis to generate super resolved actin images captured simultaneously with receptors. Future experiments will

Jak Grimes

Talk Title: Insights gained from single-particle tracking of GPCR-beta arrestin dynamics in live cells

Our group uses optical methods, which allow us to investigate GPCR signalling in living cells with high spatiotemporal resolution. We find that GPCRs undergo complex interactions with their signalling partners and structural elements of the plasma membrane, resulting in the formation of dynamic signalling nanodomains. These increase the speed of GPCR signalling, while allowing GPCRs to produce local effects and, thus, are likely to play a crucial role in controlling both efficiency and specificity in GPCR signalling. A thorough understanding of the critical interactions involved in the formation of GPCR nanodomains will be crucial to understand the pathogenesis of diseases such as heart failure and develop innovative therapeutic approaches.

This project focuses on using single particle tracking to study GPCRs, arrestins and clathrin-coated pits (CCP) concurrently on the plasma membrane of living cells. Whereas initially thought to exclusively mediate desensitisation/internalisation, b-arrestins have been subsequently shown to promote G-protein-independent signalling, mainly via recruiting ERK and other MAPK elements to active receptors. Very recent studies have further challenged the textbook model by suggesting a possibly new modality of catalytic b-arrestin activation, whereby transient receptor-b-arrestin interactions might induce b-arrestin signalling at CCPs without the activating receptor. However, the sequence of events involved in b-arrestin recruitment and signalling remain largely unknown.

Our results show that b-arrestin lateral mobility on the seemingly without an accompanying receptor. We find that short-lived, fast-diffusing receptor-b-arrestin complexes are replaced after stimulation by longer-lived, virtually immobile ones, apparently located both at and outside CCPs. When testing a family of adrenergic receptors with increasing b-arrestin affinities – we find that receptors are trapped at CCPs correlating with arrestin affinity, but that all receptors can trap arrestin to a similar degree. Lastly, when using a panel of b-arrestin mutants, we can selectively disrupt the ability for arrestin to bind at CCPs, and find that these molecules stay mobile even after stimulation. Interestingly these arrestin molecules, which do not accumulate on CCPs, still have the capability to trap receptors on CCPs. We are now building mathematical models to identify the most common mechanisms by which receptors and arrestins interact, to generate a lifetime of b-arrestin on the plasma membrane in living cells.

Foteini Damaskinaki (Fay)

Talk Title: Development and applications of fluorescent ligands for GPVI

Cardiovascular diseases are the leading cause of death and disability globally. Arterial thrombosis is one of the major pathophysiological states amongst them, which can lead to myocardial infarction and ischaemic stroke, especially in patients with atherosclerosis. Although there are antithrombotic drugs in the market, they have an intrinsic ability to prolong bleeding times and cause internal haemorrhage. Patients with GPVI depletion and show a mild bleeding disorder phenotype with normal coagulation data, making GPVI antagonists candidates for safe antithrombotic medication.

Receptor GPVI is the central collagen Ig-like immunoreceptor in platelets, which mediates firm adhesion through activation of integrin receptors and subsequent platelet activation and thrombus formation. It is activated by collagen, fibrin and fibrinogen, but its activation process and receptor conformation has been a subject debate for the past few years.

The purpose of this study is the generation of fluorescent ligands for GPVI to investigate its activation process and clustering pattern by microscopy techniques and its ability to distinguish between endogenous ligands. For the purposes of this study, a structure-based pharmacophore was developed from the GPVI crystallized ectodomain and was used for virtual screening of a local library of >84.000 compounds. 2000 of them were docked in GPVI using Glide (Maestro, Schrodinger) and 30 compounds were physically screened using a light transmittion platelet aggregation assay. Three compounds inhibited collagen-induced aggregation but not TRAP or rhodocytin induced ones. A competition ELISA and an NFAT-lusiferase assay were used to determine the mode of action of those molecules. From the ELISA experiments, collagen binding of a soluble wild type construct GPVI was not prevented, but the fluorescent signal in GPVI overexpressing cells was reduced in a concentration-dependent manner. These preliminary data indicate that those compounds could act as non-competitive GPVI antagonists, but more binding assays will be employed to confirm binding. Subsequent co-crystallization of GPVI and the ligands, in the University of Nottingham, will reveal their binding site and aid the design and synthesis of new fluorescent ligands for GPVI.

Jack Yule

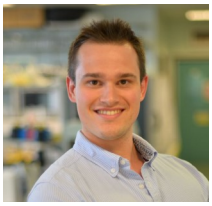
Talk Title: Investigating a Gly138Val variant in GPIba and its potentially atypical role in inherited bleeding.

Disorders of platelet count or function lead to the presentation of bleeding symptoms, which vary in nature and severity dependent on the molecular cause. Through the Genotyping and Phenotyping of Platelets (GAPP) study, a panel of atypical variants in the GPIb-IX-V complex have been identified, which may have a potentially novel role in bleeding.

Whereas typical GPIba variants result in loss of complex expression on the platelet surface, the affected patients have normal expression levels. These variants also present as heterozygous, in contrast with the typical recessive disorders that dominate GPIba-linked bleeding, such as Bernard-Soulier syndrome.

The project aims to characterise a full and integrated panel of platelet- and GPIb-IX-V function and localisation assays in order to investigate the mechanism by which these variants might contribute to bleeding.

This presentation gave an insight into the platelet function testing of a p.G138V (het) patient in which aggregation responses, platelet spreading and adhesion to physiological surfaces under flow conditions were assayed. It also highlighted the possibility for introduction of these variants into cell lines for further study, using CRISPR-Cas9 mediated editing into endogenously expressed GPIb-IX-V. It is hoped that this model will allow for receptor localisation study. Another prospective part of the project moving forward is the purification of recombinant GPIba in order to look at structural and ligand binding effects of these variants.



Eddy Wragg

Talk Title: Exploring the haemodynamic effects of Adenosine A2 receptor ligands in conscious, freely moving rats

Adenosine is involved in the regulation of every organ system, including the cardiovascular, nervous, and immune systems. Adenosine signals through adenosine receptors, of which there are four types, the A1, A2A, A2B, and A3 receptors. Both of the A2 receptors have been shown to have roles in pathological conditions, including cancer, and thus are targets for novel treatments. Knowledge of the effects that potential therapeutics have on the cardiovascular system is essential, both to assess the safety of the drug, and to gain insights into its effect on the body. Our aim is to find out the haemodynamic effects of A2A and A2B receptor agonism in conscious, freely moving rats.

In my presentation, I discussed experiments characterising the effects of the A2A receptor-selective agonist, CGS21680, and the A2B receptor-selective agonist BAY60-6583. Our results show that A2A agonism causes an increase in heart rate, a decrease in mean arterial pressure, and an increase in the vascular conductance of the arterial beds situated in the hindquarters, which is indicative of vasodilations in the resistance arteries found in this regional bed. Also, we have found that A2B receptor agonism causes an increase in heart rate and an increase in the vascular conductance of the arterial beds situated in the renal and mesenteric regions.

Looking forward, I plan on exploring if A2A or A2B receptor agonists could be utilised as potential novel antihypertensives. I will be exploring if A2 receptor agonists could help reduce the on-target hypertension caused by the receptor tyrosine kinase inhibitor, sunitinib- an anticancer drug currently used in the clinic.

COMPARE Publications

Bradley SJ, et al (2020). Biased M1-muscarinic-receptor-mutant mice inform the design of next-generation drugs. Nat Chem Biol. doi: 10.1038/s41589-019-0453-9. Epub

Drakopoulos A, Koszegi Z, Lanoiselée Y, Hübner H, Gmeiner P, Calebiro D, Decker M, (2020). Investigation of Inactive-State κ Opioid Receptor Homodimerization via Single-Molecule Microscopy Using New Antagonistic Fluorescent Probes. J Med Chem. doi: 10.1021/acs.jmedchem.9b02011. [Epub ahead of print]

Haining EJ, Lowe KL, Wichaiyo S, Kataru RP, Nagy Z, Kavanagh DP, Lax S, Di Y, Nieswandt B, Ho-Tin-Noé B, Mehrara BJ, Senis YA, Rayes J, Watson SP, (2020). Lymphatic blood filling in CLEC-2-deficient mouse models. Platelets. doi: 10.1080/09537104.2020.1734784. [Epub ahead of print]

Jullé D, Gondin AB, Von Zastrow ME, Canals M, (2020). Opioid pharmacology under the microscope. Mol Pharmacol. doi: 10.1124/mol.119.119321. [Epub ahead of print]

Khan AO, et al (2020). Surface Chemistry-Dependent Evolution of the Nanomaterial Corona on TiO2 Nanomaterials Following Uptake and Sub-Cellular Localization. Nanomaterials doi: 10.3390/nano10030401

Lane JR, Abramyan AM, Adhikari P, Keen AC, Lee KH, Sanchez J, Verma RK, Lim HD, Yano H, Javitch JA, Shi L, (2020). Distinct inactive conformations of the dopamine D2 and D3 receptors correspond to different extents of inverse agonism. Elife. doi: 10.7554/eLife.52189. [Epub ahead of print]

Seabright AP, Fine NHF, Barlow JP, Lord SO, Musa I, Gray A, Bryant JA, Banzhaf M, Lavery GG, Hardie DG, Hodson DJ, Philp A, Lai YC, (2020). AMPK activation induces mitophagy and promotes mitochondrial fission while activating TBK1 in a PINK1-Parkin independent manner. FASEB J. doi: 10.1096/fj.201903051R. [Epub ahead of print]

Wen G, Vanheusden M, Acke A, Valli D, Neely RK, Leen V, Hofkens J, (2020). Evaluation of Direct Grafting Strategies via Trivalent Anchoring for Enabling Lipid Membrane and Cytoskeleton Staining in Expansion Microscopy. ACS Nano. doi: 10.1021/acsnano.9b09259. [Epub ahead of print]

Williamson DJ, Burn GL, Simoncelli S, Griffié J, Peters R, Davis DM, Owen DM, (2020). Machine learning for cluster analysis of localization microscopy data. Nat Commun. doi: 10.1038/s41467-020-15293-x