COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

Team Science Newsletter

Edition 3 , September 2019

Team Science Committee

As this academic year draws to a close, we can reflect on the successes of COMPARE over the past twelve months, knowing we still have the upcoming Annual Symposium in September to look forward to. It is the highlight of the year within COMPARE, giving opportunities to the early career researchers to showcase their fabulous work.

As a member of the outgoing committee, I thought I'd give a rundown of some of the achievements from COMPARE Team Science this year. It is worth mentioning that this year saw the start of the joint-PhD programme within COMPARE and it is good to see these six researchers form a collaborative cohort which has developed and grown with Team Science values at heart. With this, there have also been more research links between the two universities, with more members making the most of the facilities available across both sites.

The Team Science seminars resumed with researchers having the opportunity to present their work at the other university. The scope was expanded from last year to include Prof. David Hodson, representing the PIs. Myself, Dr. Sam Cooper, and Ngoc Vo Thi (Kate) were able to present our work in Birmingham, and gained much from discussions after our talks. All the presentations were well attended, which bodes well for the future of the seminar series. We are also pleased to announce Dr. Alexander Kondrashov (University of Nottingham) will be presenting the final Team Science seminar of the year at 1pm on 31st October in the IBR Seminar Room at the University of Birmingham.

This year also saw the continuation of the Team Science Summer Studentships, building upon the great success of the programme last year. It was encouraging to see an increase in the number of proposals sent in. This is definitely a good thing for Team Science, developing the skills of both the student and supervisor! Be sure to look out for the fantastic research carried out by these young investigators at the Annual Symposium. Along with the studentships, several collaborative grants were awarded from the Team Science budget. Some of the outcomes (or ongoing results) from these collaborations are included in the following pages, with more to follow as projects progress. We are still trying to push the Team Science Slack as a forum for COMPARE discussions, so please join in the discussions at www.tinyurl.com/joinCOMPARETeamScienceSlack

The majority of the focus for the committee this year was on the Away Day. In May 2019 we hosted our second Team Science Away Day in a function room opposite Nottingham Castle. Sadly, the castle was under renovation and covered in scaffolding, but we made up for the lack of views by a great location: directly above a pub! As a committee, we decided to focus on developing leadership and management skills. With this in mind, we reached out to the European Laboratory Research and Innovation Group (ELRIG), a non-profit organisation which bridges industry and academia. Through collaboration with Del Tresize, we were able to put together a fantastic programme for the away day. It was good to build links between COMPARE and ELRIG, exposing new members to the strong community ELRIG has, potentially offering new avenues for collaboration and the development of new techniques. I won't go into the details on the Away Day here, as Chloe has already written a thorough report on the Away Day in the June 2019 COMPARE Newsletter, but I'm pleased to say the feedback we have had from all of you has been very positive. A particular highlight was the presentation on leadership from Dr. Steve Rees (Vice-President, AstraZeneca), emphasising the importance of self-leadership as a means to develop management and team leadership skills.

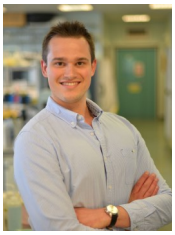
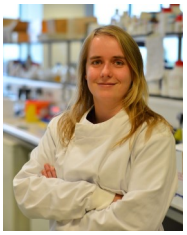
Overall, it has been another exciting year for COMPARE Team Science. As a community, it is wonderful to have the recognition from the Academy of Medical Sciences, being selected as a case study for implementing Team Science across our respective institutions. I would like to thank the rest of the committee for their outstanding work this year and for putting up with dropped/pixelated Skype calls for our meetings! I hope this coming year will continue to be successful and push even further into engaging all early career members of COMPARE in Team Science.

Mark Soave, Chair, Team Science ECR Committee 2018-2019

Team Science Committee 2019—2020

The new Team Science Committee from October 2019 will be as follows;

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Slide Deck

Thank you to everyone that has submitted their slides for the ECR Technology Spotlight, we have received 39 slides from COMPARE Early Career Researchers. This is a live document, so please submit your slides if you have not done so already. The document can be amended and updated if you feel you have learned new techniques which are appropriate.

The slides will be circulated when updated to all on the COMPARE mailing list and also be located on the COMPARE Team Science Forum workspace on Slack, please contact a team science committee member if you would like to be invited to join. For the information of those whose work is of a sensitive nature (e.g. in vivo), this document will be kept within COMPARE and not circulated externally. compare-ts-forum.slack.com

Post Funding Reports

Summer Placement - Supervisor—Andrew Benest, Student—Stephanie Caixeiro

The aim of the project aim was to understand the mechanisms that lead to the regulation of Zeb1. Stephanie became competent at primary cell culture, and manipulation using siRNA. This was then combined with droplet digital PCR, Immunocytochemistry, and several functional assays. Data generated was hypothesis driven and is hugely valuable to me, as it enables me to move to the next set of experiments (to be performed by a postdoc and future PhD students).

Stephanie will be a contributing author on a manuscript, and I am writing 3 Projects that will use Stephanie's data and will present her poster at the next COMPARE event, and has been invited back to present at a Unit team meeting soon. Steph will be given full credit as an author on her paper, and I hope she feels confident in the lab, and able to challenge her future supervisors more incoherent ideas.

Stephanie is exactly the kind of person that is needed in any dynamic laboratory environment and it was a great pleasure to haven been involved in her scientific training.

Summer Placement - Supervisor—Leigh Stoddart, Student—Omolade Otun

Ola's summer studentship placement worked really well. Ola is a talented and conscientious students who is a pleasure to work with. As Ola had completed her Master's project with me on a similar project, she could get on with experiments straight away, produce data quickly and utilise her six week project effectively. The project that Ola worked on deviated slightly from the proposed project. Using molecular biology techniques that Ola had not used before, she worked to introduce point mutations into the adenosine A2A receptor that we hypothesize are the target site of covalent labelling. In her last week we managed to do an experiment with two of these mutant receptors which gave promising results. The rest of her time she spent characterising an improved version of the fluorescent ligand which aims to transfer a fluorescent tag to the receptor. She generated some very interesting and high quality data with this ligand and it is now our favoured ligand going forward.

This project was relevant to Team Science in a number of ways. The first being that it allowed me to develop my supervisory skills further in a project that has the potential to produce a high impact publication. We believe that the data produced here is very interesting and any resulting symposium communications and publication on this data will raise both mine and COMPAREs profile within the wider GPCR community.

To complete this project ready for a high impact publication, we will have to work closely with other members of the COMPARE team to generate the relevant data. We have already enlisted the help of two other members of COMPARE to collaborate on this project. These collaborations highlight the strength of Team Science and COMPARE**.**

Ola's comments; This funding has also enabled me to gain valuable experience with techniques that had previously been completely new to me such as TR-FRET and confocal imaging. Through my masters project and summer project, I have developed an in-depth understanding of the novel ligand-directed labelled technique used. As a biochemistry student, It has greatly developed my ability to interpret and understand pharmacological data making me more confident in the lab. Moving forward, I believe this project has put me in good stead for a career in membrane pharmacology.

Summer Placement - Supervisor—Julie Sanchez, Student—Michelle (Wai) Fung

Pain dysregulation underlies major diseases, affecting 20% of the population. Opioids are still the mainstay treatments for severe acute pain. However, their use is associated with abuse and severe adverse effects. As the search for safer opioids has not been successful yet, novel strategies involving MOP signalling modulation by other membrane proteins are being investigated. TRPV1, the ion channel activated by capsaicin (the pungent component of chili peppers) has been proposed to modulate MOP regulation, however, the cellular mechanisms involving MOP/TRPV1 regulation are not yet understood and will be investigated in this project.

Activation of the TRPV1 channel with capsaicin generates a calcium influx into the cells. This influx modulates mu-opioid receptor signalling as well as other GPCRs. It prevents agonist-mediated arrestin3 recruitment to the MOP, dopamine D2 and to a lesser extent NK1 receptors and it partially inhibits G protein activation for several Gα protein subtypes (αi2/MOP, αs/D1, αq/NK1). TRPV1 activation does not influence Nanobody33 recruitment, showing that the MOP can still be activated, or mini G protein recruitment. This suggests that TRPV1 activation only modulates further downstream signalling pathways.

Michelle was a great addition to our group and she made a significant contribution to our research project. She quickly became proficient in cell culture and BRET data acquisition and interpretation. She was always interested, on time and happy to help in any way she could. This project was a valuable opportunity and I would be happy to supervise summer placement students again.

Michelle's comments

I have enjoyed working on this project as it was an interesting topic and it has been a valuable experience as I have learnt many skills in and out of the lab with great support from everyone in the team. It has given me a deeper insight in working in a research lab and has given me an idea of possibly pursuing a future in this area.

Summer Placement - Supervisor—Brad Hoare, Student—Amandeep Kaur

Amandeep's project followed on from previous summer student, who worked on a TR-FRET based method of measuring GPCR thermostability. The task was to see if the assay might work using a BRET methodology instead. Initial results were promising - we selected and optimised the concentration of a different fluorescent probe which gave a better signal. Amandeep was then able to characterise the thermostability of the CB1 and CB2 receptors in different detergent solubilising conditions and show an increase in thermostability of the receptors when they were bound to different cannabinoid ligands. This data will hopefully add further depth to the manuscript which David Tippet is currently preparing.

Amandeep presentated her data at the Cell Signalling lab meeting and will present a poster of her results at the COMPARE symposium

The funding developed my supervisory skills, as I worked to teach the background knowledge, and guide Amandeep's experiments, whilst also allowing her to develop her own independence and confidence in the lab, which gradually enabled her to make decisions about what the most sensible next experiments would be.

We have further developed and characterised a great assay which we can use in collaborative efforts which we are currently exploring. In particular, this assay can be used to rapidly identify appropriate conditions for purifying membrane proteins for structural characterisation, as well as to screen potential drug compounds for binding activity. This project has provided training and given Amandeep an experience of working in a lab doing research into the unknown. This advances the development of the 'next generation' of scientists.

Amandeep's comments: This summer project has increased my confidence in my own capabilities, and my knowledge of working in a lab. I have also been able to interact with people who are further in academia than myself, which has focussed my future career aspirations.

Summer Placement - Supervisor—David Sykes, Student—David Tippett

The COMPARE summer studentship provided to David Tippett focused upon the development of a novel FRET-based assay that allows for ultrasensitive (nanoscale) determination of protein stability and therefore requires minimal active material. The experimental work expanded upon data collected the previous summer utilising the SNAP-β2-adrenoreceptor and employing both fluorescent orthosteric tracers and a cysteine reactive dye to monitor protein unfolding.

This Thermo-FRET assay is functional in crude lysates, without the requirement of a protein purification step. It can be applied to other GPCRs and membrane proteins which contain buried cysteine residues and can be used to detect the binding of unmodified novel ligands expanding its applicability. Functionality of the solubilised receptor was readily tested using β2AR and A2AR specific fluorescent ligands by monitoring FRET between the SNAP -Lumi-Tb-labelled receptor and a bound fluorescent ligand. This assay has an additional application providing a rapid detergent screening methodology for the solubilisation of future membrane protein targets being sensitive to changes in receptor stability caused by varying detergent, buffer and ligand incubation conditions. A manuscript detaining this work is currently in production with David Tippett as lead author reflecting his invaluable contribution.

This placement addressed many of the major aims of COMPARE including the development of new procedures and models to better understand GPCR function. Secondly the development of novel fluorescent probes and methodologies (eg Thermo-FRET based technologies). Thirdly the development of novel reagents used in this case for studying receptor stability. Importantly this research has the potential for studying orphan GPCR pharmacology enabling us to identify new drugs to evaluate in future functional studies. The project has allowed us the opportunity to forge international links with other Universities, businesses and the pharmaceutical industry to explore these exciting possibilities. For example, this work has enabled the biophysical single-molecule studies of Clare Harwood in collaboration with the Tamara Miljus (group Davide Calebiro, UoB). This work has also facilitated the development of another novel Thermo-FRET based assay with Brad Hoare and his summer student Amandeep. Finally this work has implications for the 'Implementation of Team Science' as this is my first attempt at obtaining funding for academic research and my first genuine opportunity to set my own career on the 'PI track'.

Summer Placement - Supervisor—Laura Kilpatrick, Leigh Stoddart, Student—Georgia James

This summer studentship was a natural progression of work that Georgia had previously undertaken during her 3rd year MPharm lab project. Georgia tested the effect of sodium ions on the affinity of agonists, antagonists and the activity of the allosteric modulator compound 15 on the β2 adrenoceptor (β2AR) stably expressed in membranes made from HEK293 cells. The presence of sodium ions has been shown previously to themselves act as allosteric modulators at some GPCRs, therefore Georgia made 3 different buffers with varying sodium compositions. Georgia produced a robust data set for 8 ligands (4 agonists and 4 antagonists) as well as compound 15 using these 3 different buffers. Her data did not reveal any significant differences in ligand affinities with change in buffer composition, however these data were important to accurately determine any effect of the allosteric modulator itself. Although Georgia did not observe any allosteric activity for compound 15 in modifying ligand binding at the β2AR, her work will further inform future experiments using this allosteric modulator being undertaken under the umbrella of the MRC Programme grant. Georgia's work will potentially contribute to a future publication of which Georgia would be included as an author. Additionally the results of this studentship will be presented as a poster at the COMPARE annual research symposium.

Georgia gained additional laboratory experience, of techniques that she had no prior experience of in her MPharm project, in regards to aseptic technique to culture cells and making membrane preparations. These techniques will be beneficial for her future career development in any in vitro based research lab.

Both Leigh and Laura benefited from this project as the high quality and reproducible data produced by Georgia will inform further research central to the MRC Programme Grant which we are both funded by. This summer studentship gave us both additional supervisory experience which will be invaluable to our future careers, as this is a skill needing continual improvement and recognising that every student requires a tailored approach. This supervision involved managing not only laboratory teaching, but experimental planning, data analysis and data interpretation.

Georgia's comments: This summer project has been a huge opportunity for me to develop my lab skills, and gain insight into academic careers and the sheer range of work and effort that goes into medical research. Being entrusted to work somewhat independently, with the ability to organise my days, taught me a lot about my own methods of working whilst interacting with everyone working in the lab gave me insight on how to improve and has led me to consider a medical research career.

Team Science—Collaboration Grants

Collaborative Grant—Abdullah Khan, Natalie Poulter, Kellie-Rae Machlus

This project aimed to interrogate which of the known microtubule severing proteins (MTSPs) are involved in the re-organisation of tubulin in platelets and megakaryocytes.

At the University of Birmingham we will investigate the presence of and localisation of the MTSPs figetin, spastin, and the katanin sub-units using western blotting and immunofluorescence in donor platelets and induced pluripotent stem cell derived megakaryocytes. As shear stress is known to drive the formation of platelets in the bone marrow, our aim is to interrogate the recruitment of microtubule severing proteins to proplatelet protrusions generated in a microfluidic bioreactor in Dr Machlus' lab at Harvard Medical School.

This was an excellent opportunity to work with Dr Machlus' lab and combine our cell work with her microfluidic platform. This work is the basis of a collaboration which we hope will lead to the submission of a paper and grant looking at the role of microtubule severing proteins in megakaryocyte and platelets.

I gained valuable experience in working with an international collaborator and the benefit of her experience in both megakaryocyte biology and microfluidic chambers.

This has helped me establish a track record of funding for future fellowship applications, and provided me with experience in grantsmanship and working with an overseas collaborator.

ECR Publications

Bosma R1, Stoddart LA, Georgi V, Bouzo-Lorenzo M, Bushby N, Inkoom L, Waring MJ, Briddon SJ, Vischer HF, Sheppard RJ, Fernández-Montalván A, Hill SJ, Leurs R, (2019). Probe dependency in the determination of ligand binding kinetics at a prototypical G protein-coupled receptor. Sci Rep. 9: 7906

Cooper SL, Carter JJ, March J, Woolard J, (2019). Long-term cardiovascular effects of vandetanib and pazopanib in normotensive rats. Pharmacol Res Perspect. 7(3): doi: 10.1002/prp2.477

Heuninck J, Perpina Viciano C, Işbilir A, Caspar B, Capoferri D, Briddon SJ, Durroux T, Hill SJ, Lohse MJ, Milligan G, Pin JP, Hoffmann C, (2019). Context-dependent signalling of CXC chemokine receptor 4 (CXCR4) and atypical chemokine receptor 3 (ACKR3). Mol Pharmacol. doi: 10.1124/mol.118.115477. [Epub ahead of print]

Kavanagh DPJ, Lokman A, Neag G, Colley A, Kalia N, (2019). Imaging the injured beating heart intravitally and the vasculoprotection afforded by haematopoietic stem cells. Cardiovasc Res. doi: 10.1093/cvr/cvz118. [Epub ahead of print]

Khan AO, Maclachlan A, Lowe GC, Nicolson PLR, Al Ghaithi R, Thomas SG, Watson SP, Pike JA, Morgan NV; UK GAPP Study Group, (2019). High-throughput platelet spreading analysis: a tool for the diagnosis of platelet- based bleeding disorders. Haematologica. 2019 Jun 20. pii: haematol.2019.225912. doi: 10.3324/haematol.2019.225912. [Epub ahead of print]

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