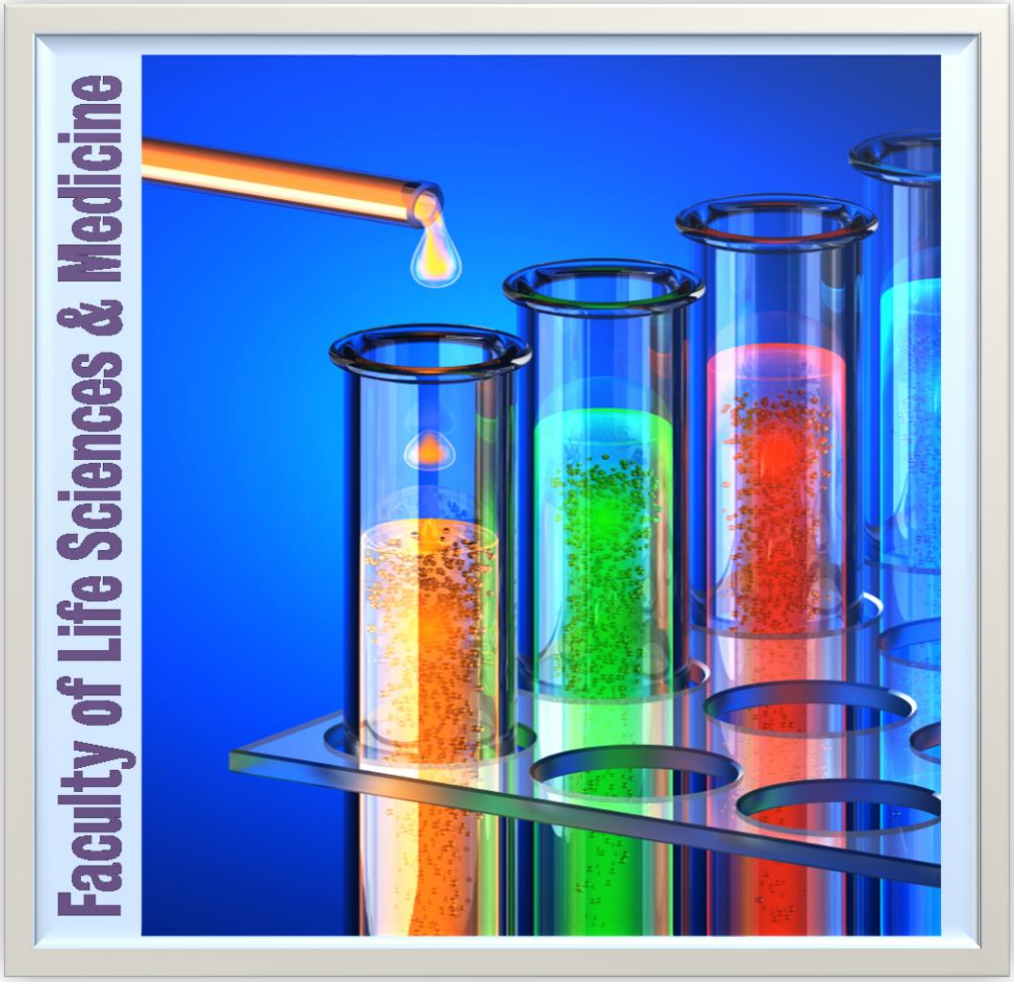
MSc /MRes Biomedical and Molecular Sciences Research

&

MSci Biochemistry and MSci Molecular Genetics

2024/2025 Research Projects



Module Organizers:

Prof Stephen Sturzenbaum

Dr Grace Walden

Research Projects

MSc/MRes Modules 7BBBM112, 7BBBM113 and 7BBBM114

MSci Module 7BBB0404

The research project is a major component of the programme and equates to approximately 6 months (MSc/MSci) or 9 months (MRes) full-time work (about 4 months (MSc/MSci) or 7 months (MRes) in the lab, and the remaining time should be spent on reading the literature, data analysis and writing the dissertation). It is therefore important that you chose a project that suits your immediate interest(s) and stimulates you sufficiently to do well.

A list of projects has been selected from research active labs within the Faculty of Life Sciences & Medicine. For your convenience, a short summary of each project has been provided including key references. Please identify SEVEN projects in the order of your preference and submit your ranked choices via the project selection tab in KEATS (MSc 7BBBM113, MRes 7BBBM114 or MSci 7BBB0404). The allocation will be based on availability, and optimized to ensure that the maximum number of students get one of their top choices.

Timetable:

MSc/MRes/MSci:

- **October 7th:** Project Titles distributed. 3:00pm – 5:00pm (FWB G.1.16)
- **October 8th – October 18th** (if necessary) discuss options with programme co-ordinator
- **October 21th:** submit project preference (rank **7** projects) via KEATS, using the selection link available on the 7BBBM113/7BBBM114/7BBB0404 KEATS pages
- **October 28th:** Announcement of preliminary allocation
- **October 28th – November 8th**: contact prospective supervisor and/or (if needed) discuss options with programme co-ordinator. Please provide a 1 page CV and short (no more than ½ page) statement/rational to the programme co-ordinator and prospective supervisor describing why you wish to do this project.

MSci:

- **14/11/24:** start experimental work. You should write up sections as you go along (Introduction, Methods, Results, Bibliography), leaving only the Discussion and Abstract to complete after the end of experiments.
- **No later than 10/3/25:** complete a *2* page outline of your dissertation and discuss the structure with your supervisor.
- **No later than 14/4/25:** draft dissertation to project supervisor for comment and correction. Your supervisor is only obliged to comment on one draft and will be instructed
- **5/5/25:** deadline for submission of dissertation.

MSc:

- November – December 2024: Start literature search.
- **Week starting 13th of January 2025 (or later, depending on your workshop choices):** start project work. You should write up sections as you go along (Introduction, Methods, Results, Bibliography), leaving only the Discussion and Abstract to complete after the end of experiments.
- **No later than 19/6/25:** submit formative Laboratory Performance Review.

- **No later than 21/7/25:** draft dissertation to project supervisor for comment and correction. Your supervisor is only obliged to comment on one draft and will be instructed not to comment on more than 2 draft versions.
- **4/8/25**: deadline for submission of dissertation.

MRes:

- November – December 2024: Start literature search.
- **Week starting 13th of January 2025 (or later, depending on your workshop choices):** start project work. You should write up sections as you go along (Introduction, Methods, Results, Bibliography), leaving only the Discussion and Abstract to complete after the end of experiments.
- **No later than 19/6/25:** submit formative Laboratory Performance Review.
- **4/8/25:** deadline for submission of *Brevia* paper
- **No later than 10/11/25:** draft dissertation to project supervisor for comment and correction. Your supervisor is only obliged to comment on one draft and will be instructed not to comment on more than 2 draft versions.
- **1/12/25:** deadline for submission of dissertation.

Matt Wilson Scholarship

Thanks to a generous donation from the family of Matt Wilson, a former student of the Biomedical and Molecular Science Research MSc we are now in a position to offer research enhancement scholarships. To celebrate Matt's legacy, the scholarship sets out to enhance MSc/MRes/MSci projects by providing a supplement towards the consumables cost of up to seven projects (5x £2500 and 2x £5000). The award can support costly experiments that would not be possible otherwise or perhaps might allow a student to travel to a collaborator to use a specialist piece of equipment. The details of the selection process will be discussed at the tutorial (7/10/2024).

Projects 1 - 34

are suitable for:

ALL students

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Research Project -1-

Supervisor(s): Professor Joseph Bateman	**E-mail (lead supervisor):** joseph_matthew.bateman@kcl.ac.uk

Project title: Investigating the role of mitochondrial stress signalling in neurodegeneration

Research area (keywords): Neuroscience, mitochondria, signalling, metabolism

Research skills (keywords): Drosophila, behaviour, imaging

Faculty/School/Department: IOPPN/ Neuroscience/ Basic and Clinical Neuroscience

Location: Maurice Wohl Clinical Neuroscience Institute, Denmark Hill campus

Project description:

Mitochondria are key regulators of cellular homeostasis and mitochondrial dysfunction is strongly linked to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Mitochondria communicate their bioenergetic status to the cell via mitochondrial stress signalling. Mitochondrial stress signalling is a pathway that enables cells to respond to changes in the functional state of mitochondria. We have shown that mitochondrial stress signalling regulates neuronal function in Drosophila models of disease. This project will investigate the mechanism by which mitochondrial stress signalling regulates neuronal function and neurodegeneration using Drosophila. Components of the stress signalling pathway will be manipulated in vivo in the nervous system to elucidate their role in neuronal function. Neuronal function will be analysed using behavioural experiments and confocal imaging.

The project will provide exciting new insights into neuronal function and generate knowledge that could lead to novel treatments for neurodegenerative diseases. Previous MSc students from this course in our lab have co-authored publications and continued to PhDs.

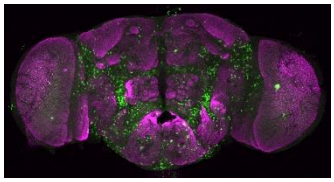

Figure 1. ATF4 (green) activation in the brain in a Drosophila mitochondrial disease model.

For more details on the lab see: https://www.kcl.ac.uk/people/joseph-bateman

Key techniques / transferable skills: The project will involve a combination of Drosophila genetics, behavioural analysis, confocal microscopy and image analysis. The student will be instructed in these techniques by experienced researchers in the lab.

References:

1. L. Granat, D.Y. Knorr, D.C. Ranson, E.L. Hamer, R.P. Chakrabarty, F. Mattedi, L. Fort-Aznar, F. Hirth, S.T. Sweeney, A. Vagnoni, N.S. Chandel, J.M. Bateman (2023). Yeast NDI1 reconfigures neuronal metabolism and prevents the unfolded protein response in mitochondrial complex I deficiency. *PLOS Genetics*, 19 (7): e1010793.

2. R.J. Hunt, L. Granat, G.S. McElroy, R. Ranganathan, N.S. Chandel, J.M. Bateman (2019). Mitochondrial stress causes neuronal dysfunction via an ATF4-dependent increase in L-2-hydroxyglutarate. *J. Cell Biol.* 218: 4007-4016.

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Research Project -2-

Supervisor(s): Dr Alison Brewer	**E-mail (lead supervisor):** alison.brewer@kcl.ac.uk

Project title: : Endothelial Dysfunction in Diabetes: exploring epigenetic mechanisms

Research area (keywords): Endothelial Cell Biology, Epigenetics, DNA methylation, Diabetes

Research skills (keywords): Cell and Molecular Biology, DNA methylation assays, Microscopy

Faculty/School/Department: FOLSM; Dept of Cardiology

Location: The James Black Centre, Denmark Hill Campus

Project description:

Background

Vascular disease is a major clinical complication associated with Type 2 Diabetes. It can result in an acceleration of atherosclerosis and impaired wound healing leading to myocardial infarction, stroke and limb amputation. It is vital that the mechanisms underpinning the development of vascular disease in diabetics are understood in order to design better therapeutics. The endothelium comprises a single-cell lining of the internal surface of blood vessels and plays a crucial role in regulating vascular tone, structure and homeostasis. Endothelial dysfunction is characterised by blunted endothelial homeostatic responses such as angiogenesis and represents an early step in the pathogenesis of vascular disease associated with diabetes[1] .

There is good evidence to suggest that the high blood sugar levels (hyperglycaemia) associated with diabetes is actually the primary cause of the dysfunction of the endothelium but the molecular mechanisms which underlie the progression of the disease are not understood. However, there is a strong correlation between changes in the DNA methylation status of the genome (termed "epigenetic" changes as they do not affect the primary DNA sequence) and endothelial dysfunction associated with hyperglycaemia. DNA methylation is regulated by the opposing actions of DNA-Methyl-Transferases, which add methyl groups to CpG di-nucleotides, and Ten-ElevenTranslocation Enzymes (TETs), which facilitate their removal. **Crucially, the activities of the TETs are regulated by the metabolic and redox status of the cell, both of which are affected by exposure to high glucose[2]** .

Project Aims

This project will aim to determine the role of dysregulated DNA methylation and TET proteins in the development of endothelial dysfunction upon exposure to high glucose *in vitro*. Cultured human aortic endothelial cells (HAECs) will be used in this study. The effects of high glucose on the transcriptional and functional inflammatory responses of HAECs (such as leukocyte attraction and permeability) will be assessed. To determine the function of the TET enzymes in these processes, the experiments will be repeated in HAECs in which the expression of the different TET enzymes has been silenced (individually and in combination) *via* siRNA-mediated genetic ablation. together with functional cell assays (including permeability, leukocyte attraction and ELISAs) fluorescence microscopy, DNA methylation analysis and bioinformatics.

Techniques The project will involve a wide range of cellular and molecular biology techniques (including cell culture, transfection, DNA RNA and protein isolation, QPCR, Western blotting), together with functional cell assays (including proliferation, permeability, leukocyte attraction and cholesterol assays) fluorescence microscopy, DNA methylation analysis and bioinformatics.

References:

1. Vanhoutte P. et al., Endothelial dysfunction and vascular disease - a 30th anniversary update. Acta Physiol (Oxf) 219, 22-96, doi:10.1111/apha.12646 (2017).

2. Green H. & Brewer A., Dysregulation of 2-oxoglutarate-dependent dioxygenases by hyperglycaemia: does this link diabetes and vascular disease? *Clinical epigenetics*, *12*, 1-15.(2020).

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Research Project -3-

Supervisor(s): Ryuichi Fukuda	**E-mail (lead supervisor):** ryuichi.fukuda@kcl.ac.uk

Project title: Mechanisms of cardiomyocyte dedifferentiation and regeneration

Research area (keywords): heart regeneration, dedifferentiation

Research skills (keywords): immunostaining, high-resolution confocal imaging, DNA extraction, PCR, and gel electrophoresis

Faculty/School/Department: Faculty of Life Sciences & Medicine/ School of Cardiovascular and Metabolic Medicine & Sciences

Location: Denmark Hill, The James Black Centre

Project description: Cardiomyocyte (CM) death is a major cause of heart failure after myocardial infarction (MI). Most current therapies focus on preventing adverse ventricular remodelling after MI, but do not compensate for the loss of CMs. Recent studies have shown that adult zebrafish, neonatal mouse and pig hearts regenerate after MI, a process in which dedifferentiation of CMs prior to their proliferation is essential. CM dedifferentiation is a novel concept whereby CMs undergo phenotypic changes in morphology and gene expression to revert to earlier developmental phenotypes, allowing them to proliferate. Although CM proliferation has been extensively studied, much less is known about the mechanism regulating CM dedifferentiation. There is currently no therapeutic approach for human cardiac regeneration. Therefore, further understanding of CM dedifferentiation/proliferation and identification of novel targets for cardiac regeneration is of great importance. We have recently identified the factors that induce CM dedifferentiation and subsequent proliferation. Our preliminary data suggest that these factors modulate signalling pathways that have not been tested in gene therapy approaches for cardiac regeneration. The central aim of this proposal is to elucidate the mechanisms that regulate CM dedifferentiation and proliferation.

The project will involve:

1. **Molecular Biology Techniques:** Utilizing advanced molecular biology methods to analyze gene expression.
2. **Immunostaining:** Applying immunostaining techniques to visualize specific proteins and cellular structures in regenerating myocardial tissue.
3. **Confocal Imaging:** Employing confocal microscopy to obtain high-resolution images of myocardial cells and tissues, allowing for detailed analysis of cellular morphology and function.

Expected Outcomes:

- A comprehensive understanding of the basic principles of myocardial regeneration.
- Proficiency in molecular biology techniques, immunostaining, and confocal imaging.

References:

Zhao, Z., *et al.* Organoids. *Nat Rev Methods Primers* **2**, 94 (2022). https://doi.org/10.1038/s43586-022-00174-y

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Research Project -4-

Supervisor(s): Ryuichi Fukuda	**E-mail (lead supervisor):** ryuichi.fukuda@kcl.ac.uk
Project title: The latest CRISPR/Cas9 genome editing technology	
Research area (keywords): CRISPR/Cas9, genome editing, Molecular Biology, Cell Culture	
Research skills (keywords): CRISPR/Cas9, human cell culture, DNA extraction, PCR, and gel electrophoresis, vector construction	
Faculty/School/Department: Faculty of Life Sciences & Medicine/ School of Cardiovascular and Metabolic Medicine & Sciences	
Location: Denmark Hill, The James Black Centre	

Project description:

Background: CRISPR/Cas9 technology has revolutionized the field of genetic engineering, offering unprecedented precision and efficiency in gene editing. This technology holds immense potential for future applications across various fields, including medical research, gene therapy, cancer treatment and tissue engineering. The importance of this technology will only continue to grow in the future, but gaining a thorough understanding of the theoretical knowledge and mastering the practical skills takes time.

Project Overview: This project aims to equip graduate students with a comprehensive understanding and hands-on experience of fundamental CRISPR/Cas9 techniques in human cell culture models. A student will systematically learn essential skills in molecular biology and cell culture, which are critical for successful gene editing experiments.

Objectives:

1. **Master CRISPR/Cas9 Basics:** Gain a thorough understanding of the CRISPR/Cas9 system, including its components, mechanisms, and applications.
2. **Molecular Biology Techniques:** Develop essential basic skills in molecular biology techniques such as DNA extraction, PCR, and gel electrophoresis.
3. **Cell Culture Skills:** Acquire cell culture techniques, including cell maintenance, gene transfection, and selection of edited cells.

Expected Outcomes: By the end of this project, a student will have a solid foundation in CRISPR/Cas9 technology, molecular biology, and cell culture techniques. This knowledge will prepare them for advanced research and applications in genetic engineering and related fields.

References:

Mazhar Adli, The CRISPR tool kit for genome editing and beyond, Nature Communi, **9**, Article number: 1911 (2018)

DOI: 10.1038/s41467-018-04252-2

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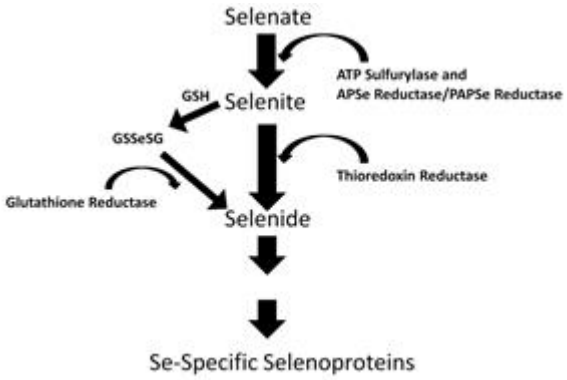
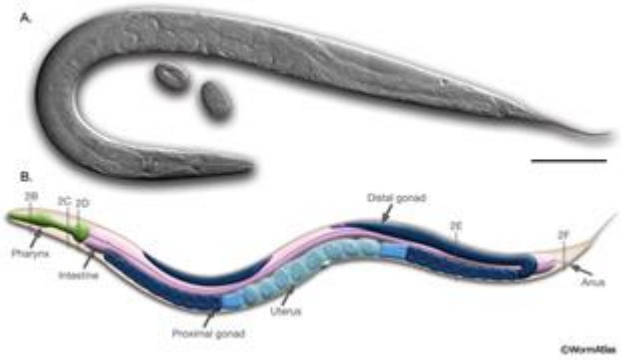
Research Project - 5 -

Supervisor(s): Prof. Stephen Stürzenbaum, Alex Dyson, Tong Kang	**E-mail (lead supervisor):** stephen.sturzenbaum@kcl.ac.uk
Department: Faculty of Life Sciences & Medicine, King's College London	
Location: Franklin Wilkins Buildings, Waterloo Campus, Lab 3.123	
Project title: How to live long(er): Impact of reduced selenium species on the genetic phenotype of *C.elegans*.	
Research area: molecular genetics, oxidation-reduction biochemistry, ageing biology	
Keywords: *C. elegans*, Selenium, ageing, qPCR, RNAi, phenotypic endpoints	

Project description:





The ability of reduced chalcogen species (e.g., sulfide [S], selenide [Se]) to induce 'suspended animation' has intrigued the medical community and beyond. Sulfide is a putative therapeutic in several disease areas and is able to extend lifespan and healthspan in *C.elegans*, one of the premier models of aging. Selenium shares some chemical and biological characteristics with sulfur, is an essential micronutrient incorporated into redox active selenoproteins that protect against oxidative stress, and is associated with longevity. Selenium levels decline with age and elevated in centurions however, the impact of modulating selenium abundance on redox status and life/healthspan is yet to be addressed experimentally. This project will address this shortfall by investigating to what extent reduced selenium species (e.g., sodium hydroselenide; NaHSe) exert an impact on ageing in *C.elegans.* The project will utilize state-of-the-art molecular biology techniques such as RNA knockdown by RNAi, target gene specific transcriptomic analysis by qPCR and genetic background screens using mutant nematodes. This will entail training in transferable skills that go beyond the scope of worm research. Past students from our lab have contributed to and co-authored 6 papers and we hope to continue this trend! For more information, please see our lab website http://www.toxicogenomics.info/

References: [1] Qabazard B, Li L, Gruber J, Peh MT, Ng LF, Kumar SD, Sturzenbaum, SR et al. Hydrogen Sulfide Is an Endogenous Regulator of Aging in *Caenorhabditis elegans*. Antioxidants & Redox Signaling. 2014 Jun;20(16):2621–30. [2] Cupp-Sutton K, Ashby M. Biological Chemistry of Hydrogen Selenide. Antioxidants. 2016 Nov 22;5(4):42. [3] Samra K, Kuganesan M, Smith W, Kleyman A, Tidswell R, Arulkumaran N, et al. The Pharmacology and Therapeutic Utility of Sodium Hydroselenide. IJMS. 2021 Mar 23;22(6):3258. [4] Newton TD, Pluth MD. Development of a hydrolysis-based small-molecule hydrogen selenide (H 2 Se) donor. Chem Sci. 2019;10(46):10723–7.

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Research Project -6-

Supervisor(s): Rushad Pavri	**E-mail (lead supervisor):** rushad.pavri@kcl.ac.uk

Project title: Investigating the function of proteins regulating antibody somatic hypermutation

Research area (keywords): Antibodies, B cells, somatic hypermutation,

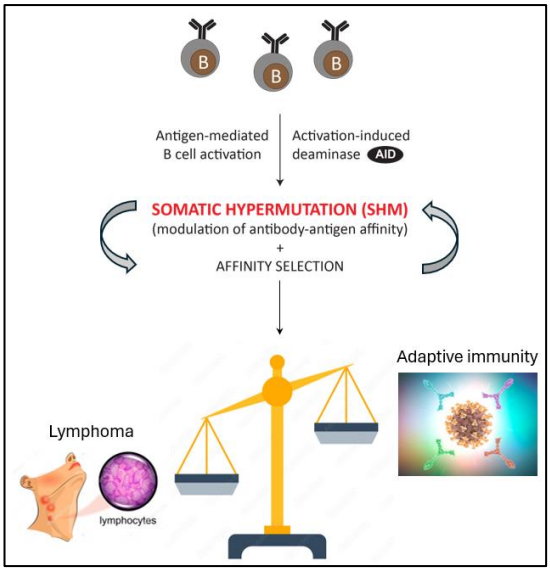
Research skills (keywords): Molecular biology, generation and characterization of cell lines with CRISPR, chromatin immunoprecipitation, data analysis and critical thinking skills.

Faculty/School/Department: FoLSM/SIMS/Peter Gorer Dept. of Immunobiology

Location: Guy's Hospital, 5th floor

Project description:

The production of a diverse repertoire of protective antibodies by B lymphocytes is the basis of all long-term adaptive immunity against pathogens. At the molecular level, this diversity results from targeted mutagenesis of the antibody-encoding immunoglobulin genes by somatic hypermutation (SHM), which is initiated by the enzyme, activation induced deaminase (AID).



In our lab, we aim to understand the molecular mechanisms of SHM, which occurs via the complex interplay of 3D chromatin structure, epigenetic changes and transcriptional regulation. An overview of projects in the lab can be found here: https://www.kcl.ac.uk/research/pavri-group.

The project offered for the MSc/MRes/MSci thesis is part of a larger, collaborative effort in our group to understand the mechanism of action of specific epigenetic and transcriptional proteins in SHM which we have recently identified via CRISPR-based genetic screens. We have established mouse models and cell-based assays for this purpose which will be integral to the project. The student will work closely with postdoctoral scientists, learn to design experiments and to critically analyse data, and be expected to develop their part of the project independently.

Methods: The project will have a strong focus on: (1) Molecular biology, especially the generation and characterization of transgenic cell lines via cloning, CRISPR editing and RT-qPCR. (2) Analysis of SHM via lentiviral production and infection of B cells, flow cytometry and sequence analysis. (3) Chromatin immunoprecipitation to study genomic occupancy of proteins and epigenetic marks.

References:

(1) Tambe et al. *Frontiers in Immunology* (2024). DOI: 10.3389/fimmu.2024.1407470. PMID: 38863710

(2) Costea et al. *Molecular Cell* (2023). DOI: 10.1016/j.molcel.2023.01.014. PMID: 36736317

(3) Fitz et al. *Nature Genetics* (2020). DOI: 10.1038/s41588-020-0605-6. PMID: 32251373

(4) Peycheva et al. *Science* (2022). DOI: 10.1126/science.abj5502. PMID: 36108018

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Research Project -7-

Supervisor(s): Konstantinos Theofilatos, Rafael Oexner, Ajay Shah	**E-mail (lead supervisor):** konstantinos.theofilatos@kcl.ac.uk

Project title: Data-centric approaches to facilitate cardiovascular disease risk stratification – improving on "one size fits all"

Research area (keywords): Cardiovascular Disease, UK Biobank, Omics, Risk Prediction

Research skills (keywords): Data Science, R, Python, Machine Learning, Bioinformatics, Statistics

Faculty/School/Department: SCMMS

Location: Denmark Hill Campus

Project description:

The cardiovascular disease (CVD) spectrum includes several highly prevalent conditions with major implications for both individual and public health care. The most effective way to alleviate the massive disease burden is primary prevention, with very efficient tools being cheaply available (e.g. antihypertensives or statins). Within the UK, such efforts are currently delivered via the NHS health check. This public health screening for middle-aged UK inhabitants entails an interview, physical and blood measurements ("clinical risk assessment"). The screening despite being expensive is limited in the number of measured features, and as a consequence has limited predictive potential.

Within the group, we have access to the UK Biobank study (UKB) dataset, a massive prospective cohort study that started enrolling patients and collecting a broad range of data (particularly rich genetic and phenotypic datasets) as early as 2006. For all participants, UKB provides ongoing electronic health and death record follow-up to date. Previous work of the group has analysed this dataset for cardiovascular endpoints (e.g. coronary artery disease, ischemic stroke, peripheral artery disease), using a range of confounders[1]. Of importance to this project, UKB provides metabolomic and genomic information coupled with rich self-answered questionnaires for n > 450,000 participants. Our preliminary data demonstrates that data-centric approaches at scale (integrating multi-omic datasets) facilitate the improved identification of individuals at risk along a cardiovascular disease spectrum.

Within this project, we will (I) comparatively assess different machine learning techniques to generate risk prediction models, (II) utilize different dimensionality reduction techniques to reduce the number of features to measure, and (III) deploy a combination of heuristic techniques (Evolutionary Optimization, Particle Swarm Optimization, Genetic Programming) for the optimization of the questionnaire based risk assessment predictors (simplification and personalization of questionnaires, optimization of accuracy and reproducibility). In conclusion, this project will therefore facilitate further development of state-of-the-art risk assessment techniques for primary prevention, with imminent potential for patient benefit at population scale.

This project will provide a unique opportunity for students who look to engage with biomedical data science and state-of-the-art machine/deep learning. Student will be trained in basic coding concepts, data science and statistical analysis using Python and R, both highly versatile and widely used bioinformatic tools in the field. Prior coding experience is not required, but we do expect a high level of intrinsic motivation and independent problem-solving. Student will be provided with the required computing infrastructure. This project will be co-supervised by Dr. Rafael Oexner (rafael.oexner@kcl.ac.uk) and Dr. Konstantinos Theofilatos (konstantinos.theofilatos@kcl.ac.uk) who are happy to discuss details with interested students.

References:

1. Oexner RR, Ahn H, Theofilatos K, Shah RA, Schmitt R, Chowienczyk P, Zoccarato A, Shah AM. Serum metabolomics improves risk stratification for incident heart failure. European Journal of Heart Failure. 2024 Apr;26(4):829-40.

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Research Project -8-

Supervisor(s): Dr Elisa Martelletti and Prof. Karen Steel	**E-mail (lead supervisor):** elisa.martelletti@kcl.ac.uk; karen.steel@kcl.ac.uk

Project title: Crosstalk between the immune and auditory systems within the cochlea.

Research area (keywords): Hearing Loss, Inflammation, Neuroscience.

Research skills (keywords): Mouse genetics; Managing mouse colonies; PCR for genotyping; Isolation of murine tissues (i.e. inner ear, brain); Gene expression study (RT-qPCR or ddPCR), Flow Cytometry (immune cell quantification); Confocal microscopy and post-acquisition analysis; Data analysis, report writing, oral presentations.

Faculty/School/Department: IoPPN, School of Neuroscience, Wolfson Sensory, Pain and Regeneration Centre

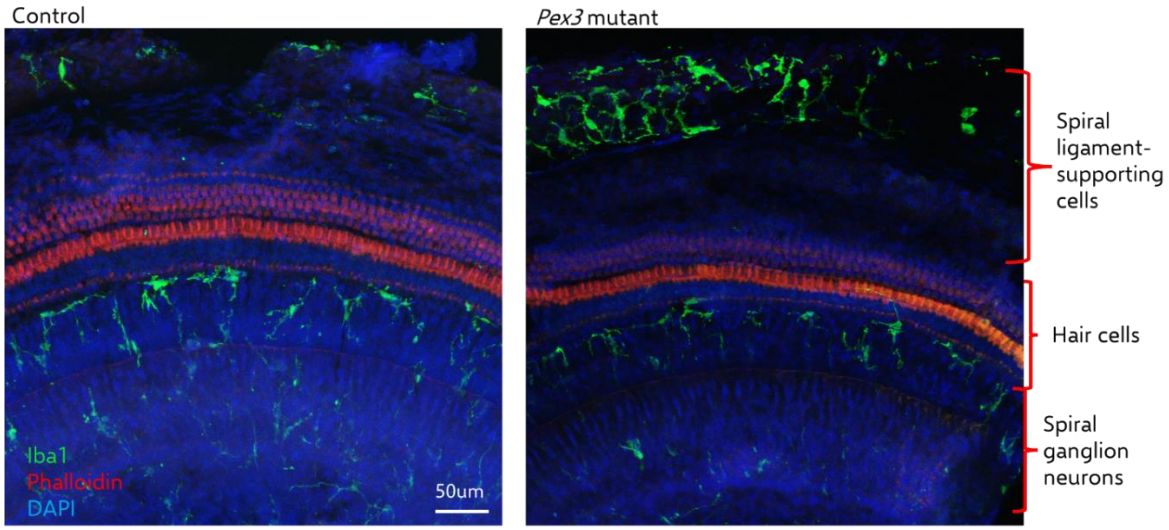
Location: Wolfson Sensory, Pain and Regeneration Centre, Guy's campus.

Project description:

Traditionally, the inner ear was considered immune-privileged, but recent evidence suggests otherwise, with immune cell activation, especially macrophages, and inflammatory responses implicated in various types of hearing loss (e.g., noise, age, drugs, genetics). However, the role of cochlear immunity in hearing physiology and hearing loss remains unclear.

This project is focused on investigating whether the cochlear inflammatory response (IR) is a primary cause or secondary effect of progressive hearing loss (HL) using *Pex3* mutant mice which were previously characterised showing progressive HL (Kochaj, Martelletti et al. 2022). Pex3 is a peroxisomal membrane protein involved in peroxisome formation and integrity, and peroxisomes have a role in the IR and phagocytosis by macrophages.

In this project, the level of key pro-inflammatory and anti-inflammatory cytokines will be measured using quantitative PCR and the cochlear immune cells will be quantify using flow cytometry. Additionally, the activation state and location of cochlear macrophages will be assessed using specific antibodies, confocal microscopy and post-acquisition analysis. If the IR is a secondary effect of the HL, the activation of macrophages, the cochlear immune cell population and level of cytokines should be the same between mutants and littermate controls before the onset of HL. If not, the IR may be contributing to the progression of HL.



Cochlear macrophages are labelled in green using Iba1 antibody.

References: Kochaj, R.M.; Martelletti, E.; Ingham, N.J.; Buniello, A.; Sousa, B.C.; Wakelam, M.J.O.; Lopez-Clavijo, A.F.; Steel, K.P. The Effect of a Pex3 Mutation on Hearing and Lipid Content of the Inner Ear. Cells 2022, 11, 3206. https://doi.org/10.3390/cells11203206

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Research Project -9-

Supervisor(s): Dr Joaquim Nunes Vieira	**E-mail (lead supervisor):** joaquim.nunes_vieira@kcl.ac.uk

Project title: Investigating the molecular determinants of blood vessel growth in the heart

Research area (keywords): Cardiovascular development and regeneration, Mouse Genetics

Research skills (keywords): Tissue microdissection, immunostaining, confocal imaging

Faculty/School/Department: FoLSM, School of Cardiovascular and Metabolic Medicine & Sciences

Location: BHF Centre of Research Excellence-James Black Centre, Denmark Hill Campus

Project description: Coronary artery disease (CAD) remains a major cause of mortality. Available therapies include for instance stenting to achieve re-vascularisation, yet many patients are not responsive. A greater understanding of vascular expansion would, therefore, assist in establishing the initiating events of coronary sprouting, and might lead to the development of therapies to encourage or modulate tissue vascularisation in the heart. Acquired expression of the transcription factor (TF) WT1 is common in the vasculature of cancers and endothelium in the infarcted heart consistent with proangiogenic functions. **Hypothesis**: WT1 regulates induction of endothelial cell (EC) sprouting required for growing adequate vascular networks and supporting organ function. To test this hypothesis, the *Aplnr-CreERT2* mouse will be used, the only existing model targeting solely sinus venosus (SV)-derived ECs. This strain will be crossed with $Wt1^{flox}$ mice to generate $Aplnr\text{-}CreERT2;Wt1^{fl/fl}$ embryos and hearts harvested between E11.5 (onset of SV-EC sprouting) and E18.5 (coronary remodelling). We will combine confocal microscopy with immunostaining (wholemount, sections) for WT1, cardiac troponinT (myocardium), cleaved caspase3 (apoptosis), EdU (proliferation), pan-endothelial PECAM1, endomucin (vein/capillary EC), FABP4 (coronary EC), arterial SM-MHC, and SOX17, a marker of activated endocardium to assess potential compensation from Endocardium-to-EC transition in *Wt1* SV-EC KO. Quantitative assessment of coronary expansion (dorsal vs ventral; outer vs inner myocardial wall), including EC sprout number, vessel length and branching density will be done using IMARIS. As a functional readout of vessel formation and adequate myocardial oxygenation, we will assess tissue hypoxia by injecting Hypoxyprobe at E15.5 and analysing labelling of SV-derived regions in KO *vs* controls. To study global heart growth and formation of coronary arteries stems, we will employ high-resolution episcopic microscopy that generates 3D surface-rendered models and digital volume data, enabling quantitative analyses. Moreover, *ex vivo* (mouse SV explants) and *in vitro* (human iPSC-EC) assays of EC sprouting will used in *Wt1* loss- and gain-of function studies to further define TF's function. This project will provide novel molecular insights on blood vessel growth, which may contribute to the development of innovative approaches to therapeutically modulate this process in disease settings where vascularisation is needed (e.g. CAD) or must be prevented (e.g. tumour growth and dissemination).

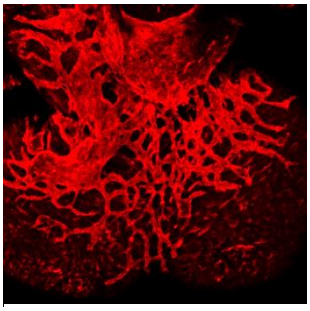

Figure 1. Coronary blood vessel expansion (anti-EMCN antibody; red) along the outside surface of the heart, the epicardium.

References:

-Cahill TJ (…) **Vieira JM*** & Riley PR* (2021) Tissue-resident macrophages regulate lymphatic vessel growth and patterning in the developing heart. Development 148(3):dev.194563. PMID:33462113 *(senior authorship).

-**Vieira JM** et al. (2017) BRG1-SWI/SNF-dependent regulation of the Wt1 transcriptional landscape mediates epicardial activity during heart development and disease. Nature Communications 8:16034. PMID:28737171

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -10-

Supervisor(s): Ryuichi Fukuda	**E-mail (lead supervisor):** ryuichi.fukuda@kcl.ac.uk

Project title: Advanced Human Organoid Technology

Research area (keywords): Human Organoids, Molecular Biology, human stem cells

Research skills (keywords): Organoid generation, human stem cell culture, DNA extraction, PCR, and gel electrophoresis, immunostaining, high-resolution confocal imaging.

Faculty/School/Department: Faculty of Life Sciences & Medicine/ School of Cardiovascular and Metabolic Medicine & Sciences

Location: Denmark Hill, The James Black Centre

Project description: Human organoid technology represents a breakthrough in biomedical research, providing a three-dimensional in vitro model that closely mimics the structure and function of human organs. This technology has immense potential for understanding human development, disease modelling, drug testing and personalised medicine. As the fields of biomedical science, tissue engineering and new therapeutic approaches continue to evolve, the importance of learning about organoid technology cannot be overstated.

Project Overview: This project aims to provide graduate students with a comprehensive understanding and hands-on experience in the latest cardiac organoid technologies. Participants will systematically learn essential skills in organoid generation, molecular biology, cell culture, tissue engineering, and analytical techniques.

Objectives:

1. **Master Organoid Creation:** Gain a thorough understanding of the principles and techniques involved in creating organoids, including human stem handling.
2. **Molecular Biology Techniques:** Develop proficiency in molecular biology techniques such as DNA/RNA extraction, PCR, and gene expression analysis.
3. **Cell Culture Skills:** Acquire essential cell culture techniques, including cell maintenance, differentiation, and 3D culture systems.
4. **Analytical Techniques:** Apply advanced analytical techniques to assess organoid development, function, and response to treatments, such as immunostaining and high-resolution confocal imaging.

Expected Outcomes: By the end of this project, participants will have a solid foundation in organoid technology, molecular biology, cell culture, tissue engineering, and analytical techniques. This knowledge will prepare them for advanced research and applications in biomedical sciences and related fields.

References:

Zhao, Z., *et al.* Organoids. *Nat Rev Methods Primers* **2**, 94 (2022). https://doi.org/10.1038/s43586-022-00174-y

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -11-

Supervisor(s): Sasi (Maria R) Conte	**E-mail (lead supervisor):** sasi.conte@kcl.ac.uk

Project title: Evaluating the role of the Soluble Epoxide Hydrolase (sEH) in mitochondrial function

Research area (keywords): Cell biology, mitochondria

Research skills (keywords): cell biology, metabolomics

Faculty/School/Department: Life Sciences and Medicine, Basic and Medical Bioscience School, The Randall Centre for Cell and Molecular Biophysics.

Location: Guy's Campus

Project description:

The Soluble Epoxide Hydrolase (sEH) is a key enzyme that modulates the metabolism of regulatory epoxy fatty acids (EpFAs). EpFAs are able to prevent mitochondrial dysfunction, to reduce subsequent ROS formation and to block resulting cellular signaling cascades. EpFA bioavailability is reduced by the action of sEH which therefore limit their beneficial effects. We have discovered a new inhibitory mechanism of sEH, whereby human sEH is allosterically modulated by endogenous electrophilic lipids binding away from the catalytic site, specifically modifying two cysteine residues, C522 and C423. These include a prostaglandin (15dPGJ2) and nitro fatty acids, whose endogenous levels may in principle be regulated with diet.

Evidence linking the effect of sEH on mitochondria function, particularly in disease states such as ischemic reperfusion injury and diabetic nephropathy is growing. In this project we aim to gain a clearer assessment of the effects of sEH inhibition on the overall lipid metabolism and mitochondria function. This will elucidate the roles of sEH in these cardiovascular diseases.

We will evaluate the mitochondria function and lipidomic profile of cardiomyocytes with and without functional epoxide hydrolase activity (e.g. with appropriate mutations) and with cells treated with a well-established sEH inhibitor, AUDA. We will also culture cells in conditions of glucose and oxygen stress. Metabolomic analysis will be conducted using LC MS/MS and NMR-based methodologies, taking advantage of the latest exciting developments for NMR lipidomics analysis and of the NMR infrastructure. Mitochondrial function will be determined by assessing morphology, oxygen consumption and lipid peroxidation with the expertise available at the Randall Centre. Mitochondrial dysfunction will be measured by assessing change in mitochondrial DNA (MtDNA) copy number and mitochondrial mRNAs.

Methodologically, the student will carry out several of the following: basic cell biology, NMR lipidomic and metabolomics analysis (including sample preparation, data acquisition and statistical analysis). Training will be provided in these areas. NMR work will be supported by dedicated personnel.

References: P.J. Meikle et al, Pharmacology & Therapeutics (2014) 143:12–23, **2.** A.A. El-Sherbeni et al. Arch. Toxicol. (2014) 88:2013–2032. **3.** J.D. Imig et al. Physiol Rev (2012) 92:101-130. **4.** J. Li, et al., Prog. Lip. Res (2017) 68:37-56. **5.** G. Abis, et al, Comm Biol (2019) 2:188. **6.** G. Abis et al., Anal. Chem (2019) 91:14865-14872. **7.** Qiu at al., (2022) J. Mol. Biol. 434:167600. **8.** Inceoglu et al., (2017) Prostagl. Lip. Mediat. 133:68-78.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -12-

Supervisor(s): Sasi (Maria R) Conte	**E-mail (lead supervisor):** sasi.conte@kcl.ac.uk

Project title: Structure-function investigations of the La-related proteins (LARPs)

Research area (keywords): Structural Biology, Biophysics, Chemistry, RNA biology, RNA binding proteins, protein-RNA interactions

Research skills (keywords): Biochemistry, structural Biology, molecular biology, Protein expression and purification, biophysical techniques (ITC, NMR, CD, MST, X-ray crystallography).

Faculty/School/Department: Faculty of Life Science and Medicine/School of Basic and Medical Biosciences/Randall Centre for Cell and Molecular Biophysics

Location: Guy's Campus

Project description

Protein-RNA interactions control the fate of mRNAs in the cell and therefore regulate protein synthesis at the translational level. It might be possible to say that almost no area of biology or biomedicine is untouched by the science emanating from understanding the regulation of gene expression at the level of mRNA translation. Nonetheless, the number of protein-RNA complexes deposited in the protein data bank (PDB) remains scarce, making it impossible to decipher a recognition code for RNA-protein interaction. Accordingly, computational prediction of RNA binding sites for proteins remains unachievable.

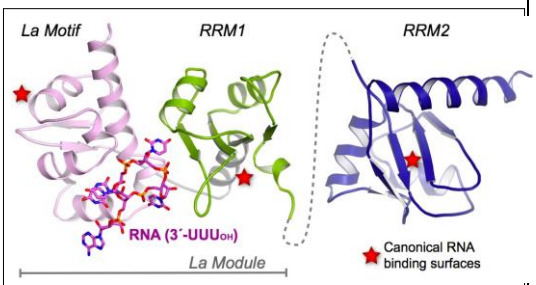

Fig. 1: *Structure and RNA interaction of human La obtained from our studies.*

An interesting example RNA binding proteins involved in regulation of gene expression has emerged recently with the identification of the superfamily of La-related proteins (LARPs). LARPs have only begun to be characterised, but it is already clear that they are involved in a wide variety of essential cellular processes including muscle differentiation, development, cell motility and cytoskeletal remodelling. LARPs also play prominent roles in many diseases: mutations can lead to autoimmunity, muscle and fibroproliferative disorders, cancer and viral infections. The common feature of LARPs is the RNA binding unit called the 'La-module', formed by pairing a La motif and an RNA recognition motif, which was first characterised in our work on the La protein, the archetypal LARP (Fig. 1). Despite the high sequence conservation in the RNA binding domain, surprisingly LARPs recognise very different RNA targets in order to perform their different function, but the molecular basis of such RNA recognition remains elusive.

The aim of the project to investigate LARP structures and mode of interactions to RNA targets in order to: (i) drive forward the studies of their cellular function; (ii) clarify their role in diseases; (iii) contribute to the understanding of the essence of protein-RNA binding specificity. This work will be conducted in parallel with groups investigating the cellular functions of LARPs.

References: 1. Cruz-Gallardo, at al. (2019). *Nucleic Acids Res., Epub, doi: 10.1093/nar/gkz144.* Recommended by F1000; 2. Maraia RJ et al. (2017) *Wiley Interdiscip Rev RNA*. doi: 10.1002/wrna.1430; 3. Lizarrondo et al (2021) RNA Biol. doi: 10.1080/15476286

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MSci Biochemistry and MSci Molecular Genetics

Research Project -13-

Supervisor(s): Agi Grigoriadis **Sasi (Maria R) Conte**	**E-mail (lead supervisor):** agi.grigoriadis@kcl.ac.uk sasi.conte@kcl.ac.uk
Project title: Novel metabolomics targets for RNA binding proteins in cancer cells	
Research area (keywords): Cancer biology, metabolism, NMR metabolomics, RNA biology, La-related proteins	
Research skills (keywords): Experimental techniques in cancer cell biology, biochemistry/molecular biology, metabolic profiling and statistical analysis of NMR data	
Faculty/School/Department: Centre for Craniofacial and Regenerative Biology, Faculty of Dental, Oral & Craniofacial Sciences	
Location: Guy's Campus	

Project description:

Cancer cells have altered metabolism to cope with high energy demands associated with cell proliferation, migration and survival. The Conte and Grigoriadis labs have initiated a project to explore the use of metabolic profiling to elucidate the role of two RNA binding proteins, namely LARP4A and LARP4B, that have recently been shown to regulate cancer cell morphology, motility, proliferation and tumour progression. **This is one of the first links between these proteins and cancer biology.**

Larp4A and LARP4B regulate the translation of a subset of mRNAs but the full list of target RNAs in cancer cells is not yet known, nor is the mechanistic link between mRNA translation regulation and the cancer cell phenotypes. It has been proposed that a few metabolic enzymes (ARPP19, CBR1, GLUL, GPX4, GSK3, NDUFB8, NME2, OAZ2) may be the targets of LARP4B, but this remains to be confirmed.

We propose to use state-of-the-art NMR-based metabolomics analysis of LARP4A/B-depleted cancer cells to identify the metabolic and signalling pathways affected by these proteins. Methodologically, the student will prepare sample extracts from different cancer cell lines (PC3 prostate cancer cells, MG63 osteosarcoma cells) that are depleted in LARP4A and/or LARP4B using siRNA and CRISPR/Cas9 technologies. Extracts will be used to perform ^{1}H 1D and ^{1}H 2D NMR experiments which will be analysed in two ways: (1) targeted metabolic profiling, to quantify TCA cycle and glycolytic intermediates, other amino acids, NADPH/NADH, total fatty acids, intact lipids etc.; (2) Untargeted metabolic profiling for a statistical analysis of global changes. Selected dysregulated genes will be validated, and following small molecule inhibition, their metabolic profiles will be analysed and functional studies on cancer cell migration and proliferation assays will be performed. **These studies will shed light for the first time on the mechanisms underlying the cancer-related functions of LARP4A and LARP4B.**

This project provides a unique and rare opportunity for the student to utilise the brand new state-of-the-art NMR infrastructure (https://www.kcl.ac.uk/innovation/research/corefacilities/smallrf/biospectroscopy/News.aspx)

References:

Maraia RJ et al. (2017) The La and related RNA-binding proteins (LARPs): structures, functions, and evolving perspectives. *Wiley Interdiscip Rev RNA*. doi: 10.1002/wrna.1430

Coleman et al. (2023) LARP4A and LARP4B in cancer: the new kids on the block. Int J Biochem Cell Biol. doi: 10.1016/j.biocel.2023.106441.

Coleman et al., (2024) The RNA binding proteins LARP4A and LARP4B promote sarcoma and carcinoma growth and metastasis. iScience, 27:109288. doi: 10.1016/j.isci.2024.109288

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MSci Biochemistry and MSci Molecular Genetics

Research Project -14-

Supervisor(s): Ran Yan; John Maher	**E-mail (lead supervisor):** ran.yan@kcl.ac.uk john.maher@kcl.ac.uk

Project title: Novel Molecular Probes for CAR T-cell Tracking with Positron Emission Tomography

Research area (keywords): CAR T-cells, PET imaging, cancer immunotherapy

Research skills (keywords): CAR T-cell engineering, bioconjugation, PET/CT imaging

Faculty/School/Department: School of Bioengineering and Imaging Sciences

Location: 4th Floor, Lambeth Wing, St Thomas' Hospital

Project description:

Chimeric antigen receptor (CAR) based immunotherapies offer a novel and rapidly developing technology that has great potential to ameliorate human disease. One fundamental challenge in the successful development and clinical application of CAR T-cells is the need to better understand the *in vivo* behaviour of adoptively infused cells. Positron Emission Tomography (PET) imaging affords a highly sensitive and non-invasive solution to visualise the CAR T-cells in real-time and quantitatively measure the CAR T-cell whole-body distribution on a patient-by-patient basis. This project will apply state-of-art radiochemistry and nuclear imaging technology to developing a novel ^{18}F-labelled PET tracer basing on an interleukin (IL)-4 mutein, IL-4DE. The ^{18}F-IL-4DE would dynamically track the migration, persistence, proliferation, and final fate of the CAR T-cells in cancer patients. Thus, the new cell tracking probe would personalise the CAR T-cell therapies by providing early insight into their safety, mechanism of action, and therapeutic efficacy.

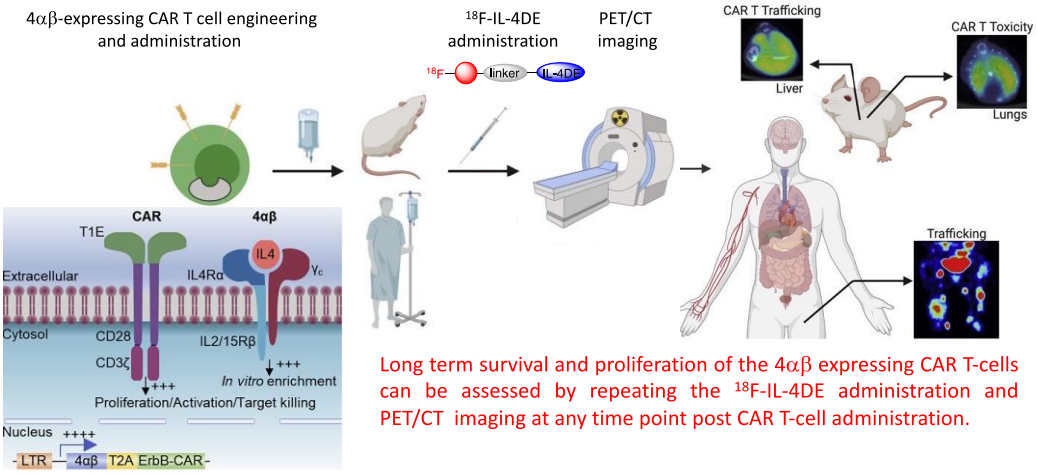

Figure 1. Illustration of novel ^{18}F-IL-4DE based PET imaging for 4αβ-expressing CAR T-cell tracking in either experimental animals or cancer patients

Techniques and skills involved in this project:

CAR T-cell engineering, cell culture; flow cytometry; tumor model development; ^{18}F-labelling; protein conjugation; *in vivo* pharmacokinetics; radiopharmaceutical biodistribution; PET/CT imaging; PET imaging and analysis.

References:

Yan, R. et al. Iodine-124 Based Dual Positron Emission Tomography and Fluorescent Labeling Reagents for In vivo Cell Tracking. Bioconjug. Chem., 2020, 31, 4, 1107–1116

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MSci Biochemistry and MSci Molecular Genetics

Research Project -15-

Supervisor(s): **Timothy Vyse & Deborah Cunninghame Graham**	**E-mail (lead supervisor):** timothy.vyse@kcl.ac.uk

Project title: Genetic variations that affect expression levels of IFN stimulated genes

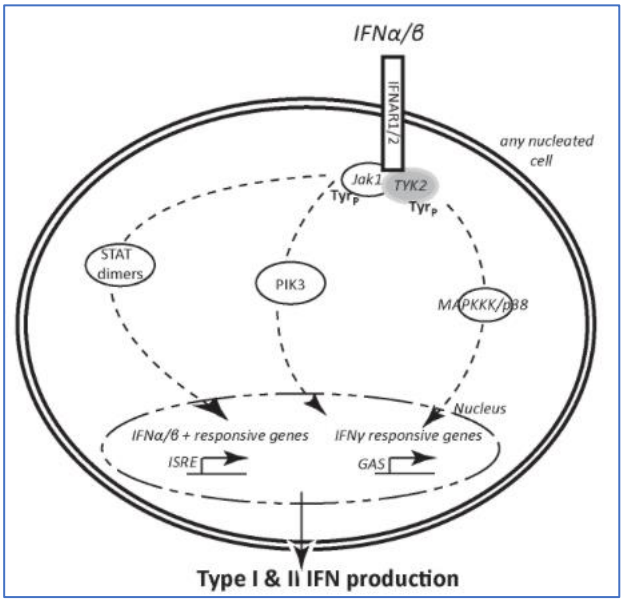
Research area (keywords): Genetics, interferon pathway, lupus

Research skills (keywords): Bioinformatics, RNA-Seq, immunology

Faculty/School/Department: FoLSM, BMBS, Medical and Molecular Genetics

Location: 7th floor Tower Wing

Background: The interferon system is a large group of molecules generating the crucial anti-viral response. Groups of these interferon responsive genes (IRG) work together in different interferon response modules to generate an interferon response. However, mounting an interferon response is not limited to situations of viral infection. In the systemic autoimmune disease, SLE, there is also aberrant upregulation of interferon response genes (IRG) (the so called "interferon signature"), in the absence of viral infection. Furthermore, large-scale genetic studies have revealed that several IRG are themselves genetic risk factors for lupus, including *IRF5, TYK2* and *STAT4*.



Type I & II IFN production

The aims of this project are:

1) Identify genetic variants within IRG which change its gene expression (cis-eQTL)
2) Calculate the IFN scores from two of the interferon response modules based on individual transcript levels (trans-eQTLs)
3) Determine whether genetic risk factors for SLE influence any of the modular IFN scores
4) Can the modular IFN scores be used to predict different sub-types of SLE?

Specific skills for the project: There will be full training provided in all aspects of the work, so the project provides a chance for the student to gain key analytical and bioinformatics skills as part of an ongoing research project underway in the ImmunoGenetics Group.

References:

El-Sherbiny, YM. *et. al* A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features *Sci. Rep* (2018) **8:** 5793.

Postal, M. *et. al* Type I interferon in the pathogenesis of systemic lupus erythematosus *Curr Opin Immunol.* (2020) **67:** 87–94.

Cunninghame Graham, DS. *et. al* Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with Systemic Lupus Erythematosus PLoS Genet. (2011) **7(10):** e1002341.

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MSci Biochemistry and MSci Molecular Genetics

Research Project -16-

Supervisor(s): Dr Godwin Aleku	**E-mail (lead supervisor):** godwin.aleku@kcl.ac.uk

Project title: **Accelerating Access To Novel Treatments For Neglected Protozoan Parasitic Diseases**

Research area (keywords): Drug Discovery, Enzymatic synthesis, Target Inhibition

Research skills (keywords): Drug discovery, enzyme Biochemistry, molecular biology, assay development

Faculty/School/Department: Institute of Pharmaceutical Science

Location: Franklin Wilkins Building

Project description:

Human parasitic protozoans cause serious human diseases, including malaria, leishmaniasis, cryptosporidium, trypanosomiasis, and toxoplasmosis, affecting millions of people, especially those from resource-constrained settings. There are extremely limited treatment options for these diseases, and drug resistance, toxicities, and cost are common barriers associated with existing treatments.[1] Effective, cheap, and safer medicines that are stable and suitable for use in resource-constrained regions could improve treatment accessibility and health outcomes.

This project explores a target-based high-throughput screening of active pharmaceutical ingredients (APIs) against validated biological targets in protozoans. Recent results from our labs have revealed that key enzyme drug targets in protozoan bind and sometimes react with a broad range of amine-containing compounds, using the amine functional group as a recognition motif. We have significant experience with enzyme systems acting on amine functional groups.

In this project, the student will exploit enzymatic synthesis to discover new compounds that can potentially treat difficult-to-treat diseases and neglected (tropical) diseases. The student will use the enzyme toolbox developed in the group to create a large library of molecules. Using our state-of-the-art analytical system such as LCMS with high-throughput capability, the exact identity of each new molecule will be confirmed. With our compound library in hand, the student will collaborate with other members of the group to screen these compounds against drug targets in protozoan.

For top-ranked hits, detailed kinetic and structural studies of enzyme-inhibitor interaction will be performed. Promising APIs will be progressed to phenotypic cell-based assays, and data obtained from the high-throughput target inhibition study will be fed to our machine learning workflow.

References:

Aleku, G. A.; France, S. P.; Man, H.; Mangas-Sanchez, J.; Montgomery, S. L.; Sharma, M.; Leipold, F.; Hussain, S.; Grogan, G.; Turner, N. J. A Reductive Aminase from *Aspergillus Oryzae*. *Nat. Chem.* **2017**, *9* (10), 961–969. https://doi.org/10.1038/nchem.2782.

Aleku, G. A. & Hollfelder F. Expanding the Repertoire of Imine Reductases by Mining Divergent Biosynthetic Pathways for Promiscuous Reactivity. 2024, in late-stage review.

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MSci Biochemistry and MSci Molecular Genetics

Research Project -17-

Supervisor(s): **Dr. Marika Charalambous**	**E-mail (lead supervisor):** marika.charalambous@kcl.ac.uk
Project title: Understanding placental transport of vital nutrients in pregnancy	
Research area: Development, metabolic biology, placenta.	
Faculty/School/Department: Department of Medical and Molecular Genetics, School of Basic and Medical Sciences	
Location: Guy's Campus, Tower Wing. Francis Crick Institute.	
Keywords: Pregnancy, lipids, obesity, development, placenta.	

Viviparity is a fundamental biological process used by mammals to protect their offspring during the vulnerable period of early life. A crucial adaptation of viviparity was the evolution of the placenta, a transient organ within which the maternal and fetal blood come into close proximity, allowing the exchange of nutrients and waste products.

Early pregnancy adaptations drive the mother to increase her appetite in order to store energy in fat depots. In late pregnancy she releases this energy and nutrients toward the rapidly growing fetus. Polyunsaturated fatty acids (PUFAs) are in particularly high demand and are essential for the development and growth of the brain and eye. PUFAs enter the body from the diet, since they cannot be synthesised by most mammals. Maternal deficiency causes blindness and neurodevelopmental disorders in humans. PUFAs are preferentially transported to the developing fetus during pregnancy, but we currently do not understand how the mother achieves this, nor how PUFAs cross the placental barrier to enter the fetal circulation.

Understanding how fatty acids are transferred from mother to fetus is important because, despite the obesity epidemic, many women are poorly nourished. Intervention strategies have increased the amount of PUFAs given to malnourished mothers in pregnancy to try to improve the health of the baby, with limited success.

Our prior studies have indicated that PUFAs are stored in maternal fat stores even prior to pregnancy, and that in the second half of pregnancy they are selectively released and travel to the liver where they are packaged into a form that can be recognised by placental cells. We have identified a candidate novel PUFA transporter (known to transport PUFAs across the blood-brain barrier) in the placenta. This project will test the hypothesis that this transporter is the route of entry into the fetal circulation.

To explore this mechanism further the student will perform immunohistochemistry and immunofluorescence studies on mouse placentas isolated from a range of pregnancy stages and with maternal obesity. This will allow us to identify the important cell types necessary for transport of this critical nutrient from mother to child.

References: 1. Haggarty, P. *Annu Rev Nutr* **30**, 237-55 (2010). 2. Hibbeln, J.R. *Lancet* **369**, 578-85 (2007). 3. Haggarty, P. *Curr Opin Clin Nutr Metab Care* **17**, 151-5 (2014). 4. Herrera, E. & Desoye, G. *Horm Mol Biol Clin Investig* **26**, 109-27 (2016).

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MSci Biochemistry and MSci Molecular Genetics

Research Project -18-

Supervisor(s):

Dr Mohammad Mahdi Karimi, Prof Agi Grigoriadis

E-mail (lead supervisor):
mohammad.karimi@kcl.ac.uk

Project title: Aberrant gene regulation in Osteosarcoma: role of retrotransposable elements

Research area (keywords): Dark Genome Biology and Bioinformatics; Bone and Cartilage Cell Biology

Research skills (keywords): qPCR, immunoblotting, RNA-seq, cell proliferation and apoptosis assays

Faculty/School/Department: Faculty of Life Sciences & Medicine, School of Cancer and Pharmaceutical Sciences, Comprehensive Cancer Centre; Faculty of Dentistry, Oral & Craniofacial Sciences

Location: Guy's Hospital & Denmark Hill campus

Project description:

Osteosarcoma (OS) remains an incurable cancer of mesenchymal origin. While changes in the coding genome have been studied in detail, how 40% of the genome comprising retrotransposable elements (RTEs) might impact OS biology is unknown. The overall aim of this proposal is to investigate the role of RTEs in gene expression perturbations in OS, validate their role in OS survival and proliferation, and explore their potential for immunotherapy.

RTEs can affect gene expression by acting as a promoter, producing chimeric transcripts between the RTE and target gene exons. The result can be either an increase in protein expression, if the ORF is not disturbed, or a decrease, if the ORF is disrupted or if translation of the chimeric mRNA is less efficient. These chimeric transcripts are readily detected with our software suite LIONS(1). In previous studies using lymphoma and colon cancer RNA-seq datasets, we detected widespread up-regulation of normally transcriptionally dormant RTEs and identified multiple genes (*IL-33* in colon cancer, *IRF5* in Hodgkin lymphoma, and *FABP7* in diffuse large B-cell lymphoma) that are recurrently up-regulated via RTE-derived promoters (2-4). **Table 1** summarises the outcome of our recent bioinformatics analysis showing the highly recurrent chimeric transcripts detected by LIONS from SAOS2, MG63, U2OS, G-292, and HOS cell lines wherein the chimeric transcripts initiate in RTEs and splice to some exons of canonical genes.

Table 2: Recurrent chimeric transcripts detected by LIONS from RNA-seq samples of OS cell lines. Chimeric Transcripts initiate in RTEs and splice to canonical genic exons.

Transcript Name	RTE class	RTE family	RTE subfamily	RTE chr	RTE start	RTE end	RTE location	Gene	Splice target	Contribution of RTE promoter to the gene expression	OS cell lines
TCONS_00121206	LINE	L1MEh	L1	chrX	152697622	152697771	Intergenic	GABRA3	exon 2	58%	SAOS2, U2OS
TCONS_00092934	SINE	AluYh3	Alu	chr5	19804143	19804331	intron 3	CDH18	exon 4	53%	HOS, SAOS2, U2OS
TCONS_00081551	SINE	AluYc3	Alu	chr3	27194122	27194414	intron 26	NEK10	exon 27	30%	HOS, U2OS

Our primary objective is to functionally validate the oncogenic and immunogenic role of these three chimeric transcripts in OS cell lines. For this purpose, we will specifically target and decrease the expression of the activated RTEs and chimeric transcript in OS cell lines by dCas9-KRAB interference (CRISPRi). The functional impact of genetic manipulation will be assessed by qPCR, immunoblotting, RNA-seq, as well as relevant proliferation, cell cycle, migration/invasion and apoptosis assays.

References:

1. Babaian A, Thompson IR, Lever J, Gagnier L, Karimi MM, Mager DL. LIONS: analysis suite for detecting and quantifying transposable element initiated transcription from RNA-seq. Bioinformatics. 2019;35(19):3839-41.

2. Lock FE, Rebollo R, Miceli-Royer K, Gagnier L, Kuah S, Babaian A, et al. Distinct isoform of FABP7 revealed by screening for retroelement-activated genes in diffuse large B-cell lymphoma. Proc Natl Acad Sci U S A. 2014;111(34):E3534-43.

3. Babaian A, Romanish MT, Gagnier L, Kuo LY, Karimi MM, Steidl C, et al. Onco-exaptation of an endogenous retroviral LTR drives IRF5 expression in Hodgkin lymphoma. Oncogene. 2016;35(19):2542-6.

4. Lock FE, Babaian A, Zhang Y, Gagnier L, Kuah S, Weberling A, et al. A novel isoform of IL-33 revealed by screening for transposable element promoted genes in human colorectal cancer. PLoS One. 2017;12(7):e0180659.

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MSci Biochemistry and MSci Molecular Genetics

Research Project -19-

Supervisor(s): Prof Snezhana Oliferenko	**E-mail (lead supervisor):** snezhana.oliferenko@kcl.ac.uk

Project title: Probing the evolution of cell biology: deciphering the impact of membrane lipids on cellular physiology in adaptive laboratory evolution experiments

Research area (keywords): Laboratory evolution, genomics, genetics, metabolism, cell biology

Research skills (keywords): Next generation sequencing, bioinformatics, molecular genetics, adaptive laboratory evolution

Faculty/School/Department: FoLSM / BMBS / Randall Centre for Cell and Molecular Biophysics

Location: New Hunt's House, Guy's Campus

Project description:

Lipid membranes are central to life and are thought to be one of the primary innovations at its origin. The organization of cellular membranes has bearing on the function of membrane-associated proteins and protein complexes, with functional implications for most biological processes, from morphogenesis, cell differentiation and cell division, to cellular communication and exploration of different ecological niches. Although membranes are an integral feature of all cells, membrane lipids vary between organisms, cell types, subcellular organelles and even different microdomains within the same membrane. We use a composite model system made of two related unicellular eukaryotes, *S. pombe* and *S. japonicus*, to investigate the processes governing membrane organization, function, and evolution.

Our lab has showed that *S. japonicus* has strikingly different membrane lipid composition and membrane physicochemical properties compared to its close relative *S. pombe*, consistent with its distinct cell biology and physiology. These differences are due to unique lipid metabolic capabilities, including those acquired from bacteria through horizontal gene transfer. The student will investigate how newly acquired genes encoding enzymes with unusual properties are integrated in cellular physiology by using a laboratory evolution approach. In adaptive evolution, we let mitotically dividing cell populations with different lipid metabolic capabilities evolve to higher fitness under specific adaptive pressures. We then sequence the genomes of evolving strains to identify relevant mutations and ask whether the evolutionary paths taken by these populations depend on membrane lipid composition. By following the genetic makeup of the evolving populations over time, we aim to reveal the genetic elements and metabolic pathways involved in adaptation.

The project involves whole-genome analyses and other bioinformatics techniques, molecular cloning, and fission yeast genetics. It can be adapted to suit the interests of the student.

References:

1. Rao, B. D., Gomez Gil, E., Peter, M., Balogh, G., Nunes, V., MacRae, J. I., Chen, Q., Rosenthal, P. B. and S. Oliferenko. **2024**. Horizontal gene transfer-initiated reorganization of lipid metabolism drives lifestyle innovation in a eukaryote. bioRxiv. doi: https://doi.org/10.1101/2024.08.21.608818
2. Alam, S., Gu, Y., Reichert, P., Bähler, J. and S. Oliferenko. **2023**. Optimisation of energy production and central carbon metabolism in a non-respiring eukaryote. *Current Biology*. 33:2175-2186. doi: 10.1016/j.cub.2023.04.046.
3. Makarova, M, Peter, M., Balogh, G., Glatz, A., MacRae, J., Lopez Mora, N., Booth, P., Makeyev, E., Vigh, L. and S. Oliferenko. **2020**. Delineating the rules for structural adaptation of membrane-associated proteins to evolutionary changes in membrane lipidome. *Current Biology*. 30:367-380

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -20-

Supervisor(s): Rocio T Martinez-Nunez, Edward J Scourfield, Varsha Kanabar-Raivadera	**E-mail (lead supervisor):** rocio.martinez_nunez@kcl.ac.uk

Project title: Development of a nanopore based sequencing approach for molecular profiling of rhinovirus.

Research area (keywords): PCR, DNA library preparation, MinION nanopore based sequencing, rhinovirus quantification

Research skills (keywords): molecular biology, cell biology, PCR, bioinformatics, nanopore NGS sequencing

Faculty/School/Department: SIMS/Dept Infectious Diseases

Location: 5th Floor Tower Wing, Guy's Hospital

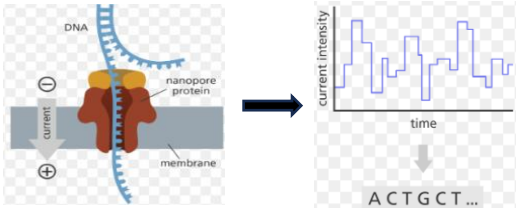
Project description:

The newly established Clinical Diagnostics Development Unit (CDDU) was launched in late 2023 as a new core facility at KCL within the Department of Infectious Diseases. It was formed off the back of the college's COVID19 testing programme(1) and its goal is to be a central unit that harmonises protocols for the processing of clinical samples from across the university, to facilitate research in infection, inflammation and immune-related disorders. We plan to offer Rhinovirus (RV) sequencing and quantification as one of our services in the near future, and seek to develop a next generation MinION nanopore-based sequencing approach for this(2).

RV is the most common cause of upper respiratory infections and causes the common cold. While in healthy people RV infection may be relatively innocuous, RV infection represents a major cause of hospitalisations in patients suffering from severe asthma and other respiratory diseases, as well as those who are immunocompromised. Sequencing of SARS CoV-2 during the COVID19 pandemic highlighted the importance of molecular profiling of pathogens for a better understanding of the pathogenicity and epidemiology across variants(3). RV is a non-enveloped, positive-sense, single-stranded RNA virus (+ssRNA virus) that can be divided into three species (RV-A, RV-B and RV-C), approximately 167 serotypes and two groups (major or minor) based on receptor usage. However, we know very little about which RVs infect which populations.

This project will aim to set up and test a new RV sequencing protocol, initially using a whole genome cDNA library based approach, whereby the candidate will first design primers to PCR amplify overlapping regions covering the entire RV genome, in a tiling approach, and then prepare and test these fragments for nanopore sequencing. If time permits, long read direct RNA sequencing may also be attempted.

In parallel, the candidate will also have the opportunity to engineer cells to stably express fluorescent proteins to aid visualisation of the cytopathic effect seen upon RV infection and further develop TCID50 assays for the quantification of RV. Together, these assays could aid in the diagnosis of RV in patients and their clinical management.



References: https://www.kcl.ac.uk/research/cddu, https://nanoporetech.com/platform/technology

1. **Reis de Andrade et al., Biology Methods and Protocols 2024**
2. **Jain et al., Nature Methods 2022**
3. **Bull et al., Nature Communications 2020**

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -21-

Supervisor(s): Dr Olena Rudyk	**E-mail (lead supervisor):** olena.rudyk@kcl.ac.uk

Project title: Investigating novel mechanisms of cell cycle regulation and its role in pulmonary hypertension and vascular remodelling

Research area (keywords): redox biology, cell cycle, cell signalling, vascular hyper-proliferative disease

Research skills (keywords): molecular biology, cell biology, metabolomics, high-throughput high-content screening

Faculty/School/Department: FoLSM, School of Cardiovascular and Metabolic Medicine & Sciences

Location: The Rayne Institute, St Thomas' Hospital, Westminster Bridge Road, SE1 7EH

Project description:

Pulmonary Hypertension (PH) is a rare but devastating vascular disease with no cure. It is recognised by high blood pressure in lung arteries and progressive right heart hypertrophy leading to rapid onset heart failure and eventual death. PH pathology is driven by progressive pseudo-malignant inward remodelling and vessel obliteration caused by the expansion of cells in all pulmonary vascular wall layers. Vascular cells isolated from the lungs of PH patients resemble similarities to cancer cells with increased proliferative potential, resistance to apoptosis, dedifferentiated phenotype, cell cycle dysfunction, adapted cellular energetics and metabolism. Despite cell proliferation being among the most significant features in PH pathology, there is a lack of detailed understanding of underlying causes or signalling pathways. Our lab investigates ROS-induced modifications of protein cysteine residues and their functional roles. Recently, we have gathered multiple *in vitro*, *in silico*, and *in vivo* evidence to elucidate a novel mechanism of cell cycle inhibition by ROS by showing that phylogenetically unique Cys135 in CDK4 is a novel allosteric site crucial for kinase activity. Perturbation of Cys135 CDK4 through oxidation to a disulfide bond with cyclin D, mutation or alkylation inhibits kinase activity. The redox state of Cys135 CDK4 is altered in human PH, an observation consistent with CDK4 being hyperactive in PH patients, further supporting the role of cell cycle dysregulation by ROS in causing PH.

The proposed project will build on these findings to further explore cell cycle regulation by ROS and its role in hyper-proliferative vascular disease. The student would be tailor-offered one or more aspects of the project, including molecular biology and biochemistry, molecular modelling, site-directed mutagenesis, cell proliferation and *in vitro* kinase activity assays that will be employed to further our understanding of the role of Cys135 as an allosteric regulator of CDK4 activity. NMR or LC/MS assessments of pulmonary vascular cells isolated from healthy donors or PH patients would provide information on how endogenous signals, such as redox state or metabolic changes, can potentiate the cell cycle and cause disease progression. Pharmacological targeting of Cys135 by high-throughput, high-content screening of a covalent fragments library in cells could be performed to identify a selective binder/s of Cys135 CDK4, imminently translatable into a novel, specific and selective therapy aiming to reverse pulmonary vascular remodelling and alleviate PH. Furthermore, functional cysteines in other cell cycle (e.g., CDK2) or transcriptional (e.g., CDK8) CDKs and their biological importance could also be investigated using established lab tools and methods.

References: PMID: 37955182; PMID: 33788196; PMID: 31186362; PMID: 35204311; PMID: 31406951.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -22-

Supervisor(s): Samantha Terry	**E-mail (lead supervisor):** samantha.terry@kcl.ac.uk

Project title: Determining the sensitivity of neuroendocrine cell lines to anti-cancer drugs in 2D and 3D models

Research area (keywords): cancer, chemotherapy, spheroid, molecular and cellular biology

Research skills (keywords): Cell culture, formation of spheroids, treatment of cells with chemotherapeutic drugs, cell survival assays, microscopy, data analysis, immunohistochemistry

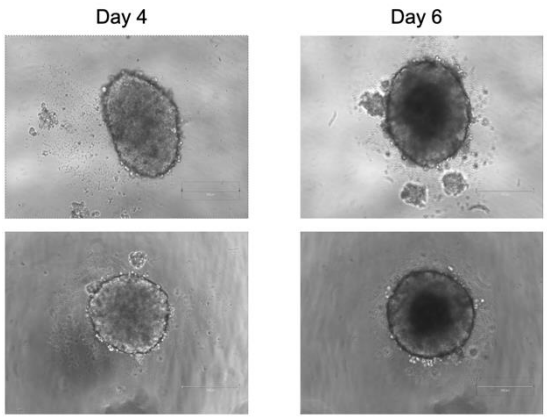
Faculty/School/Department: FoLSM/ BMEIS/ Imaging Chemistry and Biology

Location: St Thomas' Hospital

Project description:

Neuroendocrine tumours are rare but hard to treat. The aim of this project is to generate a 3D model of neuroendocrine cancer to be treated with anti-cancer agents such as chemotherapeutics. The impact of these drugs will be assessed using cell viability assays and microscopy in both 2D and 3D models. Multicellular spheroid models have demonstrated increased resistance to anti-cancer drugs in many cancer types and should be explored further in neuroendocrine tumours. This project fits within a larger piece of work looking at new combination therapy strategies for neuroendocrine tumours in 2D and 3D models, including with radiotherapy.

Picture is of cancer cells grown in spheres (spheroids).



References:

- Is It Time to Start Transitioning From 2D to 3D Cell Culture? https://www.frontiersin.org/journals/molecular-biosciences/articles/10.3389/fmolb.2020.00033/full
- 3D Cell Culture Models as Recapitulators of the Tumour Microenvironment for the Screening of Anti-Cancer Drugs https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8749977/

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -23-

Supervisor(s): Prof Christer Hogstrand, Dr Cinzia Imberti, Dr George Firth	**E-mail (lead supervisor):** christer.hogstrand@kcl.ac.uk

Project title: Zinc imaging as a biomarker for Zn^{2+} transporter-driven cell proliferation in breast cancer

Research area (keywords): zinc biology, cancer biology, radionuclide imaging

Research skills (keywords): CRISPR/Cas9, cell-based assays, radioactive uptake assays, radionuclide imaging

Faculty/School/Department: Faculty of Life Sciences & Medicine, Analytical, Environmental and Forensic Sciences, Imaging Chemistry and Biology

Location: FWB/St Thomas Hospital

Project description:

A heterodimer of zinc importers ZIP6 and ZIP10 is needed for mitosis, cell proliferation and epithelial-to-mesenchymal transition (EMT) in several cancer cells, including breast and kidney carcinomas, where it is overexpressed.[1,2,3] This presents the attractive possibility of using cellular zinc imaging as a diagnostic tool to identify malignant breast and kidney neoplasms as well as targeting ZIP6:ZIP10 therapeutically to attenuate cancer growth. Positron Emission Tomography (PET) is a diagnostic technique that permits non-invasive imaging of molecular processes in vivo by injecting patients with a radiolabelled version of a biomolecule. We have recently developed ^{62}Zn as a tool for PET imaging of zinc biology with the potential for human studies afford by total-body PET.[4,5]

This highly interdisciplinary project aims to investigate if increased ZIP6:ZIP10 mediated zinc accumulation has the potential to be a diagnostic marker of breast cancer using ^{62}Zn PET imaging.

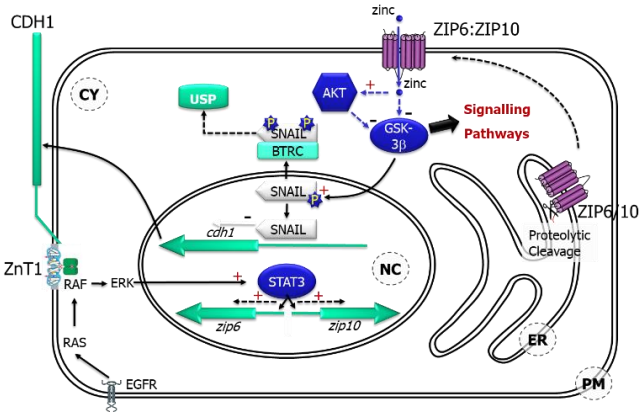


Figure 1. The ZIP6:ZIP10 heterodimer triggers EMT in breast cancer cells. Modified from Hogstrand et al. (2013)

In our previous research, we generated murine breast cancer cells with *Zip6* deleted. In the proposed project, the student will first use CRISPR/Cas9 to generate corresponding *Zip10* knockout cells as well as *Zip6*/*Zip10* double knockouts (FWB). They will then analyse cell proliferation and migration in wildtype, *Zip6* KO, *Zip10* KO, and *Zip6*/*Zip10* double knockout cells (FWB). Influx of zinc in the generated cell lines will be measured using $^{62}Zn^{2+}$ and antibodies against ZIP6 and ZIP10 will be evaluated for their abilities to block zinc import function in wildtype cells to assess their potential therapeutic efficacy in slowing carcinoma growth (St Thomas Hospital). If time allows, cell culture experiments will be followed up with animal experiments using tumour bearing mice and their ability to accumulate zinc in vivo will be imaged using ^{62}Zn PET (St Thomas Hospital).

References: 1. Nimmanon et al. (2021) 2. Taylor et al. (2016) Biochem J. 473(16):2531-443. Hogstrand et al. (2013) Biochem J. 455(2):229-37. 4. Firth et al. (2022) Metallomics. 14(10): mfac076. 5. Firth et al. (2022) RSC Chem. Biol. 3:495-518.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -24-

Supervisor(s): Dr. Qiuping Zhang, Prof. Cathy Shanahan	**E-mail (lead supervisor):** qp.zhang@kcl.ac.uk

Project title: Novel roles of nesprin-2 in cardiac function and disease

Research area (keywords): Cardiovascular, Molecular and cell biology

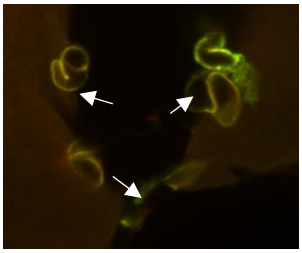
Research skills (keywords): Cell culture, in vitro mutagenesis and transfection, GST pull down and Western blot, qPCR, immunofluorescence and Imaging

Faculty/School/Department: Faculty of Life Sciences and Medicine/ School of Cardiovascular Medicine and Sciences

Location: BHF Centre of Excellence, James Black Centre, Denmark Hill Campus

Project description:

Dilated cardiomyopathy (DCM) and heart conduction defect (CD) are an important cause of heart failure and sudden cardiac death. Emerging evidence shows that mutations in proteins of the nuclear envelope (NE) LInker of Nucleoskeleton-and-Cytoskeleton (LINC) complex, which mechanically couples the nucleus to filamentous cytoskeletal networks. Nesprins-1/2 are key components of the LINC complex, highly expressed in cardiac and skeletal muscle, with mutations causative in both Emery-Dreifuss muscular dystrophy and DCM. Most recently, we have identified two novel nesprin-2 mutants in the nesprin-2 gene in a family with a strong pedigree of heart CD, preliminary data on the patient fibroblasts and muscle tissue showed nuclear morphology defect, suggesting the LINC complex disruption. Therefore, we propose to investigate if these two newly identified nesprin-2 mutants disrupt nesprin-2 interaction with its binding partners, resulting in uncoupling nucleoskeleton and cytoskeleton and defective machanotransduction; processes that may underlie the disease mechanism, which will influence the strategies for potential translational approaches. In this project, we aim to 1) examine any NE-LINC complex disruption in the fibroblasts and muscle autopsied tissues derived from the patients compared with age matched donor controls by , 2) investigate if there is any change of subcellular localisations of nesprin-2 and its binding partners in transfected neonatal rat cardiomyocytes and C2C12 myoblasts/myotubes and also disrupted interactions in these proteins using GFP and/or GST-tagged mutants.



Convoluted & clustered nuclei observed in patient muscle tissue (arrowed).

The student will receive training on primary cell culture and myoblast differentiation, *in vitro* mutagenesis and transfection, protein isolation, GST pull down and Western blot, RNA extraction and quantitative PCR, immunohistochemistry and immunofluorescence staining, confocal microscopy and data analysis.

References:

1.De Silva, S. et al. Nesprin-1: Novel Regulator of Striated Muscle Nuclear Positioning and Mechanotransduction. Biochem Soc Trans. 2023, BST20221541. doi: 10.1042/BST20221541

2.Zhou C, et al. Novel nesprin-1 mutations associated with dilated cardiomyopathy cause nuclear envelope disruption and defects in myogenesis. Hum Mol Genet. 2017; 26 (12):2258-2276.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -25-

Supervisor(s): QueeLim Ch'ng	**E-mail (lead supervisor):** queelim@kcl.ac.uk

Project title: Analysis of Insulin Regulatory Networks

Research area (keywords): Molecular Genetics, Development, Physiology, Neuroscience

Research skills (keywords): *C. elegans* Molecular Genetics, Gene Regulation, Developmental Assays, Microscopy, Statistical Analysis

Faculty/School/Department: IoPPN/Neuroscience/Centre for Developmental Neurobiology

Location: 4th Floor, New Hunt's House, Guy's Campus

Project description:

Background: Insulins and insulin-like peptides (ILPs) are hormones that regulate crucial aspects of health and disease, including development, metabolism, and ageing. Both humans and *C. elegans* have multiple ILPs, but how these ILPs cooperate in neuroendocrine networks is poorly understood. We identified a set of ILPs that regulate a switch between reproductive growth and developmental arrest that allows animals to survive harsh conditions (Fig. 1). Now we aim to elucidate how these ILPs cooperate during this process.

Aim: We will analyse the genetic and regulatory interactions between key ILPs guided by our knowledge of the ILP network. This knowledge will reveal how inputs from multiple ILPs are integrated in a physiologically important process needed for survival. In turn, we will learn about the information processing functions in the ILP network.

Approach: We will construct specific double mutants in distinct ILPs using crosses and PCR and measure their combined effects on development. Students will also analyse developmental phenotypes and use a fluorescence microscopy to measure gene expression. If this project is funded by a Matt Wilson Scholarship, we will also investigate the effects of ILPs using transcriptomics.

Training: The student will be directly trained by Dr Ch'ng in advanced genetics and molecular biology, *C. elegans* development, and quantitative fluorescence microscopy.

Figure 1. Signalling cells secrete insulin-like peptides to regulate a key developmental switch. (**Left**) Scheme for how ILPs regulate a switch between reproductive growth versus developmental arrest. (**Right**) Expression of the *ins-6* ILP in food-sensing neurons in *C. elegans*, visualised using a transcriptional reporter.

References: Fernandes de Abreu et al., (2014) An Insulin-to-Insulin Regulatory Network Orchestrates Phenotypic Specificity in Development and Physiology. *PLoS Genet*, 10(3):e1004225. doi: 10.1371/journal.pgen.1004225.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -26-

Supervisor(s): QueeLim Ch'ng	**E-mail (lead supervisor):** queelim@kcl.ac.uk

Project title: Neural Regulation of Insulin

Research area (keywords): Molecular Genetics, Development, Physiology, Neuroscience

Research skills (keywords): *C. elegans* Molecular Genetics, Gene Regulation, Developmental Assays, Microscopy, Statistical Analysis

Faculty/School/Department: IoPPN/Neuroscience/Centre for Developmental Neurobiology

Location: 4th Floor, New Hunt's House, Guy's Campus

Project description:

Background: The brain has the fascinating ability to control physiological processes in the body, including metabolism, development, and immunity. These functions are regulated by hormones produced in the brain. The brain also contains neurotransmitters that relay information between neurons. Neurotransmitters and hormones both occur in the brain, but how these two systems are connected is not known. Delineating these connections will reveal new insights into the neural control of physiological functions in the body.

Aim: We will elucidate how neurotransmitters regulate key ILPs involved in developmental switch essential for survival in harsh environments in *C. elegans* (Fig. 1). This knowledge will reveal the neural inputs into insulin pathways that underlie a developmental decision between growth versus survival.

Approach: We will test neurotransmitter pathways for roles in development and assess their combined effects in compound mutants. Students will analyse developmental phenotypes and use fluorescence microscopy system to measure gene expression. If this project is funded by a Matt Wilson Scholarship, we will also investigate the global effects of specific neurotransmitter pathways using transcriptomics.

Training: The student will be directly trained by Dr Ch'ng in advanced genetics and molecular biology, *C. elegans* development, and quantitative fluorescence microscopy.

Figure 1. Signalling cells secrete insulin-like peptides to regulate a key developmental switch. (**Left**) Scheme for how ILPs regulate a switch between reproductive growth versus developmental arrest. (**Right**) Expression of the *ins-6* ILP in food-sensing neurons in *C. elegans*, visualised using a transcriptional reporter.

References: Fernandes de Abreu et al., (2014) An Insulin-to-Insulin Regulatory Network Orchestrates Phenotypic Specificity in Development and Physiology. *PLoS Genet*, 10(3):e1004225. doi: 10.1371/journal.pgen.1004225.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -27-

Supervisor(s): **Jody Rosenblatt**	**E-mail (lead supervisor):** jody.rosenblatt@kcl.ac.uk

Project title: The role of Piezo1 in energy production

Research area (keywords): epithelial, extrusion, ATP, mitochondria, membrane potential, Piezo1

Research skills (keywords): imaging, cell biology, cell culture, molecular biology, biochemistry

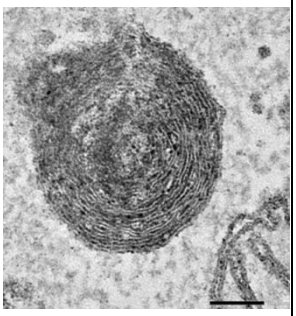
Faculty/School/Department: FoLSM/ BMBS/ Randall and Cancer Centres

Location: New Hunt's House 2.36, Guys Campus

Project description:

Metabolism lies at the heart of life and its misregulation is an important driver of disease. Many biological synthesis pathways are coupled to energy production. When cells are rapidly dividing, they consume glucose, which in collaboration with mitochondria, fuels ATP production as well as amino acid and nucleotide production, essential for cell growth and replication. As cells stop dividing and reach homeostasis, they shift predominantly to oxidative phosphorylation within mitochondria for energy production, while emphasizing amino acid production over nucleotide production. Intriguingly, destruction of mitochondria and release of cytochrome c triggers induced cell death or apoptosis, emphasizing its importance to cellular life. However, inhibition of ATP synthesis by glycolysis and mitochondria does not cause cell death, but instead induces ATP from an alternative pathway.

Here, we propose a new energy production system that might occur in post-mitotic differentiated, mature epithelial cells. We have identified a new transient organelle (see picture) that forms in epithelial cells as they age and become smaller, which resembles a lamellar body or onion-like structure potentially formed from smooth endoplasmic reticulum. We term this organelle a piezosome, since its synthesis requires the stretch-activated channel Piezo1. Mysteriously, ATP colocalizes to these piezosomes, even in cases where mitochondrial ATP production is inhibited with oligomycin A. We propose that piezosomes could produce energy and potentially lipids, as cells mature and specialize to secrete needed proteins. We first identified these organelles, as their production and destruction are essential for signalling cell extrusion the predominant mechanism driving cell death in epithelial. Thus, the piezosome could serve an ancillary role to mitochondria as cells mature and die.



The master's student will work with a postdoctoral fellow directly on this project to test this hypothesis in the following aims:

1. **Test using imaging and metabolomics if piezosomes produce ATP.**
2. **Test if disruption of piezosomes alters energy production.**
3. **Identify components of piezosomes.**

References: **1.** Voltage-dependent volume regulation controls epithelial cell extrusion and morphology. Mitchell SJ, Pardo-Pastor C, Zangle TA, Rosenblatt J. *bioRxiv.* 2023 Mar 14. doi: 10.1101/2023.03.13.532421. **2.** Mechanical stretch triggers rapid epithelial cell division through Piezo1. Gudipaty SA, Lindblom J, Loftus PD, Redd MJ, Edes K, Davey CF, Krishnegowda V, Rosenblatt J. *Nature.* 2017 Mar 2;543(7643):118-121. doi: 10.1038/nature21407. Epub 2017 Feb 15. PMID: 28199303. pdf **3.**Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Eisenhoffer GT, Loftus PD, Yoshigi M, Otsuna H, Chien CB, Morcos PA, Rosenblatt J. *Nature.* 2012 Apr 15;484(7395):546-9. doi: 10.1038/nature10999. PMID: 22504183. pdf

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -28-

Supervisor(s): Dr. Tiago Dias
Dr. Joaquim Nunes Vieira

E-mail (lead supervisor): tiago.dias@kcl.ac.uk
joaquim.nunes_vieira@kcl.ac.uk

Project title: Exploring the epicardial Slit-Robo signalling in heart development

Research area (keywords): Cardiovascular development and regeneration, Mouse genetics

Research skills (keywords): Tissue microdissection, immunostaining, confocal imaging

Faculty/School/Department: FoLSM, School of Cardiovascular and Metabolic Medicine & Sciences

Location: BHF Centre of Research Excellence-James Black Centre, Denmark Hill Campus

Project description: The molecular mechanisms guiding epicardial epithelial-to-mesenchymal transition (EMT) and epicardium-derived cells (EPDCs) function remain poorly characterised. The Slit-Robo signalling pathway, recognised by its role in axon guidance, has been linked to epicardial EMT-associated processes (e.g. AV valve development), with Slit ligands and Robo receptors showing complementary expression patterns in the developing epicardium and underlying tissue.

Hypothesis: Our hypothesis is that epicardial Slit-Robo signalling plays a role regulating EMT, setting up the subepicardial niche during normal heart development and guiding EPDCs migration to support the normal development of the heart. To test this hypothesis, we will use heart tissue collected from mice genetically modified with individual or a combination of conditional knockouts in the epicardium of the Slit-Robo ligands (Slit2 and Slit3) and/or receptors (Robo1 and Robo2) by using the Wt1CreERT2/+ driver and tamoxifen-pulsing at E9.5 and E10.5.

Workflow: The student will work directly with Dr. Tiago Dias to process the hearts harvested and assist with quantification and analysis of the data. Immunofluorescence staining of wholemount and microsections (cryostat) will be performed using antibody combinations that allow the analysis of ventricle trabeculation and compaction as well as abnormal growth and patterning of the cardiac vasculature (EMCN), lymphatics (LYVE1), and nerves (TUJ1) in the subepicardium (figure 1). Hybridization Chain Reaction (HCR) staining will also be used for specific targets. Images acquired by confocal microscopy will be quantified by a combination of semi-automated pipelines and automated machine learning. In addition, to complement the *in vivo* studies and assess EMT capabilities, heart tissue explants will be cultured *ex vivo*; and an *in vitro* mouse epicardial cell line EMT model will be used to evaluate the impact of siRNA-targeted inhibition of Slit-Robo signalling. Cell migration assays and molecular biology techniques such as RNA/DNA extraction, real-time PCR, genotyping will be performed. If keen, the student can be exposed to other techniques related to the main project, including microtissue dissection, probing of scRNAseq and ATACseq datasets, high-resolution episcopic microscopy (HREM) and others.

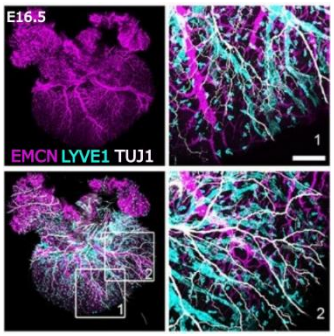


Figure 1. Immunofluorescence staining highlighting cardiac vasculature (EMCN), lymphatics (LYVE1) and nerves (TUJ1) on the surface of a E16.5 heart.

Outcomes: We expect this project to provide mechanistic insights into how Slit-Robo regulates epicardial EMT, which may contribute for the design of novel approaches in regenerative medicine aimed at leveraging the potential of the epicardium role in cardiac tissue regeneration. This research project is incorporated in a main project funded by the BBSRC and the workflow detailed is expected to contribute for a scientific publication.

References:

- Mommersteeg *et al*. (2015) Disrupted Slit-Robo signalling results in membranous ventricular septum defects and bicuspid aortic valves. DOI: 10.1093/cvr/cvv040
- Kruszka *et al*. (2017) Loss of function in ROBO1 is associated with tetralogy of Fallot and septal defects. DOI: 10.1136/jmedgenet-2017-104611

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -29-

Supervisor(s): Dr. Matthew Stroud	**E-mail (lead supervisor):** matthew.stroud@kcl.ac.uk **Website:** stroudlab.com **Twitter:** @stroud_lab

Project title: Investigating the role of the nuclear envelope in the heart

Research area (keywords): Cardiovascular, cell biology, skeletal muscle

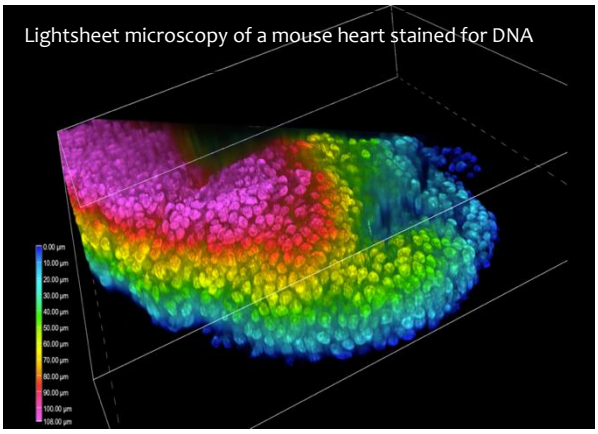
Research skills (keywords): Biochemistry, real-time qPCR, super-resolution microscopy

Faculty/School/Department: FOLSM, School of Cardiovascular and Metabolic Medicine and Sciences

Location: BHF Centre of Excellence, James Black Centre, Denmark Hill Campus

Background: Heart failure is frequently caused by changes to a family of proteins that surround the nucleus, called the nuclear envelope, and provide the nucleus with structural integrity. Surprisingly, despite the critical role of these proteins, they are largely understudied and the **molecular mechanisms leading to disease remain elusive.** Novel insights into their function have recently been revealed from studying cardiac development, during which, the heart stiffens by several orders of magnitude. Importantly, the nuclear envelope must adapt accordingly to changes in stiffness observed throughout development. **Our recent data show the essential role of a protein called Lem2 in this process, which involves heterochromatin re-organisation, nuclear envelope repair, DNA damage, and heart development.**

To test the hypothesis that **Lem2 plays an essential role in the adaptation to increases in physiological stiffness**, this project will investigate the molecular effects on cells seeded on **bioengineered substrates** that mimic physiological stiffnesses observed in the heart. **Cellular dynamics** of nuclear envelope proteins will be monitored using **state-of-the-art imaging**, along with a range of **biochemistry** and **molecular biology** approaches.



Objectives:

1) Use state-of-the-art microscopy (including super-resolution and lightsheet imaging) to reveal the effects of exercise on subcellular nuclear envelope proteins.
2) Understand the effects of exercise on LINC complex gene expression and protein levels.

Techniques/ transferable skills: This project will involve a wide range of biochemistry, molecular biology, and cell biology techniques. By the end of the project, the student will have a solid foundation in these techniques, all of which are essential for a future career path in research.

For more information about the lab: http://www.stroudlab.com/the-lab.html

References:

- **Stroud MJ**. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. *Biophys Rev* 2018;**10**:1033-1051. doi.org/10.1007/s12551-018-0431-6
- Ross JA…**Stroud MJ**[$]. Lem2 is essential for cardiac development by maintaining nuclear integrity. *Cardiovasc Res*. 2023. doi: 10.1093/cvr/cvad061

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -30-

Supervisor(s): Gilbert Fruhwirth	**E-mail (lead supervisor):** gilbert.fruhwirth@kcl.ac.uk
Project title: **Investigating the role of hypoxia in lung cancer models.**	
Research area (keywords): Cancer biology; imaging; hypoxia; cell and tissue analysis.	
Research skills (keywords): The student will perform molecular and cell biology work, grow various cells, perform different in vitro assays, help establish animal tumour models, may harvest tissues, and analyse animal tissues *ex vivo* using fluorescence microscopy and/or flow cytometry. There is the option for guided *in vivo* radionuclide imaging or transcriptomics analyses, if a Matt Wilson fellowship application were successful.	
Faculty/School/Department: Cancer and Pharmaceutical Sciences	
Location: Guy's Campus / Cancer Centre - Innovation Hub 9th floor	

Background: Cancer is a rising global problem as we extend life expectancy with lung cancer being among the deadliest cancers and presenting with limited treatment options. Tumour hypoxia is known to play a major role in rendering cancer cells resistant to various therapies and more invasive[1]. It drives various responses, importantly HIF-1α stabilisation. HIF-1α integrates also other signals, but its stabilisation is a robust and widely accepted consequence of hypoxia[2]. It protects cancer cells from hypoxia-induced necrosis.[2] While previous large-scale gene expression studies delivered hypoxia signatures, they also highlighted that cellular responses to hypoxia were heterogeneous[3]. The understanding of molecular processes underlying hypoxia-mediated lung cancer (LC) progression *in vivo* is limited. We developed a radionuclide-fluorescence reporter-based fate-mapping approach to in vivo identify LC cells that have experienced hypoxia. We can track them and identify daughter cells and aim for in-depth analysis of those such cells ex vivo.

Project proposal: Our new approach exploits hypoxia sensing based on HIF-1α and regulates permanent cell marking (with radionuclide-fluorescence reporters[4,5]). Currently, this is implemented in cancer cell models and here we aim for two advancements: (i) full characterisation of an immunocompetent lung cancer model, and (ii) implementation into a niche-labelling approach to learn about the tumour microenvironment, too. This will include work with DNA molecules, cancer cells including their characterization in vitro as well as tumour growth investigations and downstream ex vivo tissue analyses (e.g. metastatic spread, imaging reporter stability, tumour microenvironment). Analysis methods will involve several fluorescence-based methods including flow cytometry, immunofluorescence microscopy, immunohistochemistry.

There is also the option to participate in guided in vivo SPECT/CT imaging or transcriptomic analyses of cultured cells or harvested tissues (to discover differences between unswitched and switched cells) if an application to the Matt Wilson fellowship were successful.

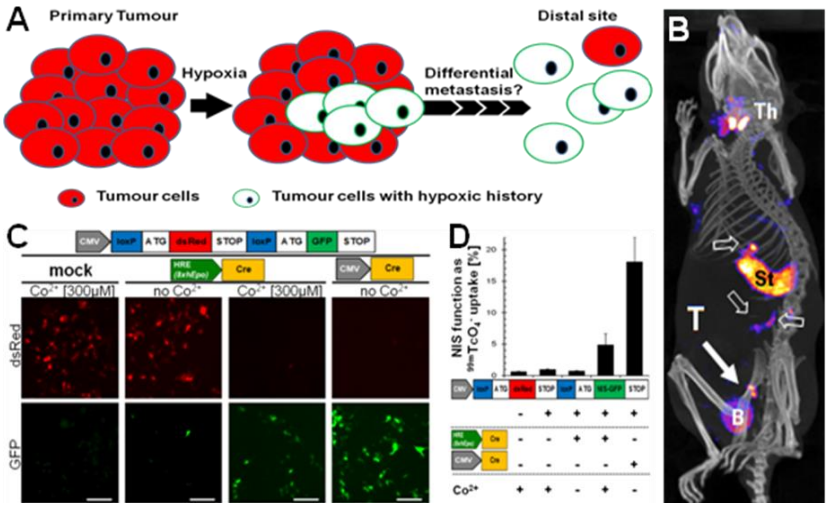


Fig. Hypoxia-responsive permanent switch reporter. (A) *Scheme showing sensor-reporter expressing cells in the primary tumour (red) that experience hypoxia and switch permanently to become green and in vivo traceable (by SPECT radionuclide imaging).* ***(B)*** *Reporter gene in vivo imaging: primary tumour (T, full arrow) and metastatic sites (open) as detected by SPECT/CT. Endogenous signals are confined to organs largely irrelevant for metastasis (thyroid/salivary glands (Th), stomach (St), bladder (B)).* ***(C)*** *The permanent switch components are the sensor (HRE>Cre) and the reporter. The reporter, switches when co-expressed in cells stably expressing HRE>Cre only under Co^{2+} challenge (a hypoxia mimic) or real hypoxia (constitutive Cre serves as positive control); scale bar 100µm.* ***(D)*** *Radionuclide reporter gene function (measured as [^{99m}Tc]TcO_4^- uptake) of cells expressing switches or controls.*

References: Lu X *et al. Clin Cancer Res* (2010) **16**:5928. **2.**Poon E *et al., Exp Rev Mol Med* (2009) **11**: e26. **3.**Chi JT *et al., PLoS Med* (2006) **3:** e47. **4.**Fruhwirth GO *et al., J Nuc Med* (2014) **55**:686. **5.**Maiques et al. *Brit J Cancer* (2021).

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -31-

Supervisor(s): Younbok Lee	**E-mail (lead supervisor): younbok.lee@kcl.ac.uk**

Project title: Exploring molecular mechanisms driven by RNA binding proteins in neurodegenerative disease for developing gene therapy

Research area: Molecular biology in neurodegenerative disease

Faculty/School/Department: Basic and Clinical Neuroscience

Location: WOHL (Denmark Hill Campus)

Keywords: ALS/FTD, RNA binding proteins, phase separation, neurodegeneration, gene therapy

Project description:

Aim: This project is to investigate the role of new neurodegenerative disease responsive RNA binding protein ARPP21. The student will study molecular mechanisms of how the mutations on ARPP21 is responsible for the neurodegenerative disease progress.

Background: Amyotrophic lateral sclerosis (ALS, also known as motor neuron disease- MND) causes progressive muscular paralysis and death due to the degeneration of motor neurons in the brain and spinal cord. Cytoplasmic aggregates of the predominantly nuclear protein TDP-43 is the hallmark motor neuron pathology in 95% of cases. We recently identified novel RNA granules that is involved in the RNA binding protein ARPP21 in familial and sporadic ALS cases. The mutations are located in the disordered and low-complexity domain and affected to the phase separation and generate insoluble cytoplasmic ARPP21 aggregates in mammalian cell lines, rat cortical neurons and human neurons derived from induced pluripotent stem (iPS) cells. The ARPP21 mutant protein causes TDP-43 to mislocalise and form insoluble aggregates that are dependent on RNA binding.

Techniques: Cell culture (cell line, iPSC lines), primary neuron culture, DNA transfection, western blot, immunocytochemistry, live cell imaging, and Human brain staining.

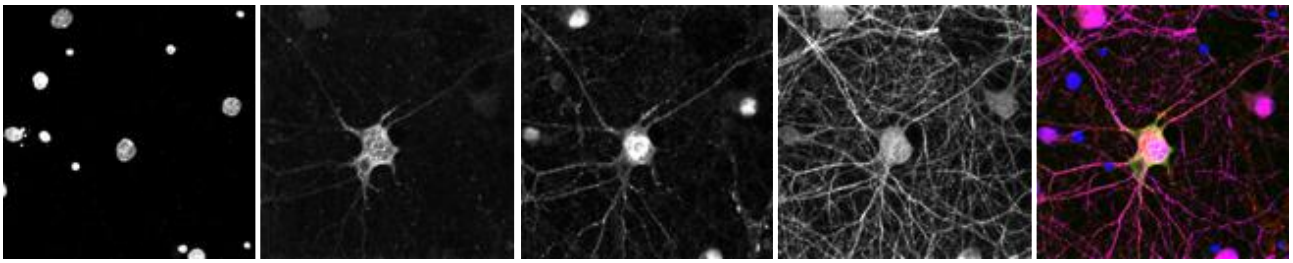


Figure 1. Neuron specific protein marker MAP2 (purple) is coimmunostained with RNA binding protein (green). Confocal microscopy is used to capture the images.

References:

[1] Stimulating VAPB-PTPIP51 ER-mitochondria tethering corrects FTD/ALS mutant TDP43 linked Ca^{2+} and synaptic defects.Acta Neuropathol Commun. 2024 Feb 23;12(1):32 [2] Mutations in FUS lead to synaptic dysregulation in ALS-iPSC derived neurons. Stem Cell Reports. 2024 Feb 13;19(2):187-195. [3] Disruption of ER-mitochondria tethering and signalling in C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia.Aging Cell. 2022 [4] Interactome screening of C9orf72 dipeptide repeats reveals VCP sequestration and functional impairment by polyGA.Brain. 2022 Apr 18;145(2):684-699. [5] Cytoplasmic TDP-43 is involved in cell fate during stress recovery. Hum Mol Genet. 2021 [6] Regulation of Synapse Weakening through Interactions of the Microtubule Associated Protein Tau with PACSIN1. J Neurosci. 2021 Aug 25;41(34):7162-7170. [7] Disease Mechanisms and Therapeutic Approaches in *C9orf72* ALS-FTD. Biomedicines. 2021 May 25;9(6):601.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -32-

Supervisor(s): Younbok Lee	**E-mail (lead supervisor): younbok.lee@kcl.ac.uk**

Project title: Investigating the molecular mechanism of RNA foci and RAN translation in C9orf72 ALS/FTD for developing gene therapy

Research area: Molecular biology in neurodegenerative disease

Faculty/School/Department: Basic and Clinical Neuroscience

Location: WOHL (Denmark Hill Campus)

Keywords: ALS/FTD, RNA binding proteins, neurodegeneration, *RNA foci, gene therapy*

Project description:

Aim: Aim: This project is to investigate the role of repeat-associated protein translation in Motorneuron disease and Dementia disease model. The student will study molecular mechanisms of how the non-canonical translation is triggered in c9orf72 mutant in vivo and in vitro.

Background: Repeat-associated translation (RAN) is found in ALS and FTD. The long nucleotide repeat GGGGCC is discovered in c9orf72 ALS/FTD, which cause nuclear toxic RNA foci and source of dipeptide protein translation through RAN translation. We have found the critical components that is binding with nuclear RNA foci which may be responsible for the disease progress in the c9orf72 ALS/FTD. Following this study, we found that the GGGGCC repeat binding protein is involved in RAN translation and possible disease progress.

Methods: Student will learn: Cell culture (cell line, iPSC lines), primary neuron culture, DNA transfection, western blot, immunocytochemistry.

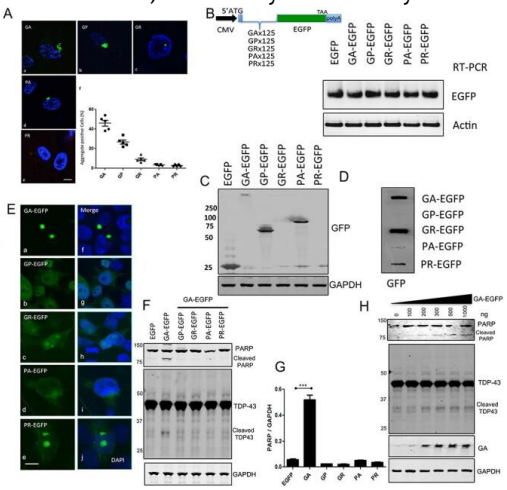


Figure 1. Poly GA is the most abundant DPR in human cortex and is toxic to cultured cells. (A) The total numbers of DPR-positive cells were counted in the C9orf72-positive human frontal cortex (n 1/4 5). (a-e) Rabbit anti-DPR antibodies were used for the immunostaining for GA, GP, GR, PA, and PR (Green). DAPI (blue) was used for nuclear counterstaining, and (f) 50 cells were counted from each section (n 1/4 5). Scale bar 1/4 5 mm. (B) Schematic diagram of scrambled synthetic DPR, which incorporated start codon ATG. Artificially synthesized DPR DNAs were cloned into an EGFP expression vector, which generates N-terminal DPR-EGFP fusion proteins. Expression of DPR-EGFPs was tested in HEK-293 cells. Transcription of mRNA levels of transfected plasmids was assessed by semi-quantitative RT-PCR. (C) Western blot analysis of total cell lysates showed that poly GA forms a high molecular weight species. (D) Filter trap assay revealed that GA-EGFP, GR-EGFP and PR-EGFP are insoluble. An equal amount of lysates was loaded on nitrocellulose membranes. Membranes were stained with an anti-GFP antibody. (E) Expression of synthetic 125 repeat DPRs in HEK-293 cells results in the formation of cytoplasmic (GA-EGFP) or nuclear inclusions (GR-EGFP, PR-EGFP). In contrast, no inclusions were detected in GP-EGFP and PA-EGFP expressing cells. Scale bar 1/4 5 mm. (F) Western blot analysis of total cell lysates from DPR-expressing cells for anti-PARP (top) and anti-TDP-43 (middle) revealed that GA-EGFP produced PARP cleavage, a marker of cell death and a 37 kDa TDP-43 cleavage product. GAPDH was used to normalize the protein loading. (G) Quantitative analysis of PARP western blotting showed that cells bearing inclusions of GA have significantly increased cleaved PARP (***P<0.0001). (H) Dose-response of GA-EGFP (50-10000 ng/well) was assessed for PARP (top) and TDP-43 (middle) cleavage. The high molecular weight of GA-EGFP (bottom) was increased following the dose of GAEGFP plasmids.

References:

[1] Stimulating VAPB-PTPIP51 ER-mitochondria tethering corrects FTD/ALS mutant TDP43 linked Ca^{2+} and synaptic defects.Acta Neuropathol Commun. 2024 Feb 23;12(1):32 [2] Mutations in FUS lead to synaptic dysregulation in ALS-iPSC derived neurons. Stem Cell Reports. 2024 Feb 13;19(2):187-195. [3] Disruption of ER-mitochondria tethering and signalling in C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia.Aging Cell. 2022 [4] Interactome screening of C9orf72 dipeptide repeats reveals VCP sequestration and functional impairment by polyGA.Brain. 2022 Apr 18;145(2):684-699. [5] Cytoplasmic TDP-43 is involved in cell fate during stress recovery. Hum Mol Genet. 2021 [6] Regulation of Synapse Weakening through Interactions of the Microtubule Associated Protein Tau with PACSIN1. J Neurosci. 2021 Aug 25;41(34):7162-7170. [7] Disease Mechanisms and Therapeutic Approaches in *C9orf72* ALS-FTD. Biomedicines. 2021 May 25;9(6):601.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -33-

Supervisor(s): Younbok Lee	**E-mail (lead supervisor): younbok.lee@kcl.ac.uk**

Project title: Exploring the molecular mechanism of granulin in c9orf72 ALS/FTD for gene therapy

Research area: Biochemistry and Molecular biology in neurodegenerative disease

Faculty/School/Department: Basic and Clinical Neuroscience

Location: WOHL (Denmark Hill Campus)

Keywords: ALS/FTD, RNA binding proteins, neurodegeneration, *RNA foci*, *gene therapy*

Project description:

Aim: Aim: This project investigates the role of granulin (GRN) for c9orf72 ALS/FTD. The student will study molecular mechanisms of how the GRN is responsible for the ALS/FTD disease progress.

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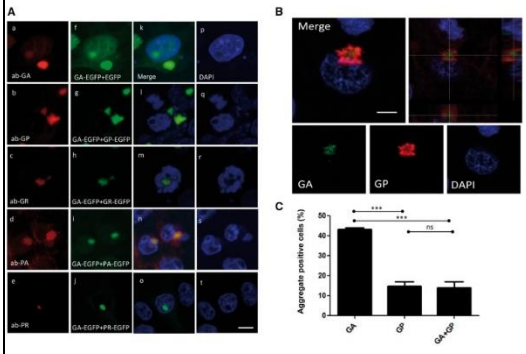
Background: Amyotrophic lateral sclerosis (ALS, also known as motor neuron disease- MND) causes progressive muscular paralysis and death due to the degeneration of motor neurons in the brain and spinal cord. Repeat-associated translation (RAN) is found in ALS and FTD. The long nucleotide repeat GGGGCC is discovered in c9orf72 ALS/FTD, which cause nuclear toxic RNA foci and source of dipeptide protein translation through RAN translation. We have found the critical components that is binding with nuclear RNA foci which may be responsible for the disease progress in the c9orf72 ALS/FTD. Following this study, we found that the GGGGCC repeat binding protein is involved in RAN translation and possible disease progress. The student will investigate the role of GRN and other RNA binding proteins to protect the toxic effect of the RNA foci and Dipeptide for C9 ALS/FTD, which will be applied for gene therapy.



Methods:

Molecular cloning, Cell culture(cell line, iPSC lines) , primary neuron culture, DNA transfection, western blot, immunocytochemistry. Human brain staining.

Figure 1. Crosstalk between RAN protein (DPRs) : GA sequesters GP. (**A**) HEK-293 cells were transfected with (GA-EGFP+EGFP, GA-EGFP+GP-EGFP, GA-EGFP+GR-EGFP, GA-EGFP+PA-EGFP and GA-EGFP+PR-EGFP). Cells were immunostained with specific rabbit antibodies against DPRs (GA, GP, GR, PA and PR; red). Green indicates EGFP-expressing DPRs and blue shows nuclear counterstaining with DAPI. Scale bar = 5 μm. (**B**) Human brain sections (temporal lobe) from C9orf72 expansion carriers were used for double-staining with poly GA (green) against poly GP (red) (*n*=3). (**C**) The percentage of poly GA and poly GP positive cells show that the majority of poly GP aggregates are positive with poly GA on hippocampal neuron (***P<0.0001).

References:

[1] Stimulating VAPB-PTPIP51 ER-mitochondria tethering corrects FTD/ALS mutant TDP43 linked Ca^{2+} and synaptic defects.Acta Neuropathol Commun. 2024 Feb 23;12(1):32 [2] Mutations in FUS lead to synaptic dysregulation in ALS-iPSC derived neurons. Stem Cell Reports. 2024 Feb 13;19(2):187-195. [3] Disruption of ER-mitochondria tethering and signalling in C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia.Aging Cell. 2022 [4] Interactome screening of C9orf72 dipeptide repeats reveals VCP sequestration and functional impairment by polyGA.Brain. 2022 Apr 18;145(2):684-699. [5] Cytoplasmic TDP-43 is involved in cell fate during stress recovery. Hum Mol Genet. 2021 [6] Regulation of Synapse Weakening through Interactions of the Microtubule Associated Protein Tau with PACSIN1. J Neurosci. 2021 Aug 25;41(34):7162-7170. [7] Disease Mechanisms and Therapeutic Approaches in *C9orf72* ALS-FTD. Biomedicines. 2021 May 25;9(6):601.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -34-

Supervisor(s): Dr. Barry Panaretou	E-mail (lead supervisor): barry.panaretou@kcl.ac.uk

Project title: Determining whether Tuberous sclerosis syndrome is caused by mutations in *TSC2* that prevent interaction of the TSC2 polypeptide with the TTC27 molecular chaperone.

Research area (keywords): Two hybrid interaction trap, site-directed mutagenesis

Research skills (keywords): PCR, Assembly of recombinants using Gibson assembly, DNA sequencing, yeast culture and transformation, two hybrid screen

Faculty/School/Department: Institute of Pharmaceutical Science, Franklin-Wilkins Building

Location: Franklin-Wilkins Building

Project description:

Tuberous sclerosis (TSC) syndrome is a disorder caused by mutations in either *TSC1* or *TSC2*, genes that encode the protein products hamartin and tuberin, respectively. The pathology of this syndrome can include mental retardation, epilepsy, as well as heart, lung and kidney defects. The *TSC1* and *TSC2* gene products form a complex that activates the GTPase activity of Rheb, thereby inhibiting the Rheb-GTP-dependent stimulation of cell growth through mTOR (the Target Of Rapamycin complex that coordinates eukaryotic cell growth and metabolism with environmental inputs including nutrients and growth factors).Specifically, Rheb is de-activated by the GTPase activation (GAP) domain at the C terminus of TSC2.

We have found that the TTC27 (TetraTriCopeptide repeat) chaperone physically interacts with the TSC2 GAP domain, inferring that TTC27 is required to fold or maintain stability of the TSC2 GAP domain. The GAP domain of TSC2 is a hotspot for missense mutations that cause TSC. Some of these mutations may cause TSC because the mutant polypeptide can no longer interact with TTC27, implying that it is loss of TSC2 stability that causes tuberous sclerosis syndrome in these patients. The objective of the project is to make these mutants and use the yeast two hybrid trap to determine if the mutant TSC2 GAP domains bind to TTC27, thereby generating some insight regarding how some of the GAP mutations cause disease.

Reference:

Li *et al.,* (2004) TSC2: filling the GAP in the mTOR signaling pathway Trends in Biochemical Sciences Vol.29 No.1 doi:10.1016/j.tibs.2003.11.007

Projects 35-73

are suitable for:

MSc students

MRes students

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -35-

Supervisor(s): Charlotte Odendall	**E-mail (lead supervisor):** charlotte.odendall@kcl.ac.uk

Project title: *Salmonella* inhibition of the host Interferon response

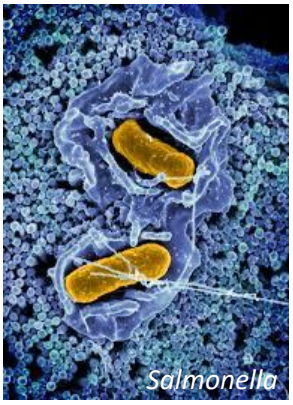
Research area (keywords): Host-pathogen interactions

Research skills (keywords): Microscopy, Flow Cytometry, RT-qPCR, Cellular microbiology

Faculty/School/Department: School of Immunobiology and Microbial Sciences, Infectious Diseases

Location: Guy's Hospital, Borough Wing, 3rd Floor

Project description: *Salmonella* Typhimurium is a major cause of bacterial gastroenteritis globally. The pathogen uses its Type III Secretion Systems (T3SS) to inject effector proteins into host cells, enabling it to evade immune responses and manipulate cellular pathways. These effectors are essential for *Salmonella* to replicate within host cells and manipulate the host immune response.



Interferons (IFNs), particularly Type I (IFN-α, IFN-β) and Type III (IFN-λ), are key components of the host immune defence against infections. Through the induction of hundreds of interferon-stimulated genes (ISGs), IFNs generate an antimicrobial state and restrict pathogen replication[1]. While *Salmonella* is known to manipulate various host immune pathways through its T3SS effector proteins, the extent to which it hijacks IFN signalling to enhance its survival remains unclear.

Our preliminary data show that IFN treatment restricts *Salmonella* replication in host cells, significantly reducing bacterial load. This raises the question of whether *Salmonella* effectors actively counteract IFN signalling to promote bacterial survival. Our published work with *Shigella*[2], another enteropathogen with similar infection modalities, has identified T3SS effectors that block IFN responses to enhance bacterial infection. Therefore, we hypothesize that *Salmonella* may employ a similar strategy. The goal of this project is to identify the specific *Salmonella* effectors that manipulate IFN signalling and to understand their mechanisms of action.

This project will systematically screen *Salmonella* effectors using an overexpression library and mutant *Salmonella* strains to uncover those that subvert IFN responses. The aims of this study are:

Aim 1: *Salmonella* effectors screens. We will use a library of *Salmonella* effector proteins and transfect epithelial cells with individual effectors. In parallel, epithelial cells and macrophages will be infected with *Salmonella* mutant strains lacking one or up to six effectors. IFN signalling will be measured using luciferase-based reporter assays. Expression of IFNs and ISGs will be assessed using RT-qPCR and western blotting.

Aim 2: Functional assays. The importance of identified effectors in promoting *Salmonella* infection will be determined with infection and replication assays. Cells will be infected with various *Salmonella* strains in the presence or absence of IFN and bacterial numbers will be determined by plating of live bacteria, flow cytometry or microscopy.

Aim 3: Mechanistic analysis of identified effectors. For effectors identified as strong modulators of IFN signalling, further mechanistic studies will be performed using biochemical assays (co-immunoprecipitation, protein interaction studies, or mass spectrometry).

References:

1- Alphonse N, Dickenson RE, Odendall C (2021) Interferons: Tug of War Between Bacteria and Their Host. Front Cell Infect Mi 11:624094. https://doi.org/10.3389/fcimb.2021.624094

2- Alphonse N, Wanford JJ, […], Odendall C (2022) A family of conserved bacterial virulence factors dampens interferon responses by blocking calcium signaling. Cell. https://doi.org/10.1016/j.cell.2022.04.028

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -36-

Supervisor(s): Dr Luigi Margiotta-Casaluci & Dr Anna Zoccarato	**E-mail (lead supervisor):** Luigi.Margiotta-Casaluci@kcl.ac.uk
Project title: Investigating the metabolic effects of the antidiabetic drug metformin in human and zebrafish liver cells using high-content methodologies.	
Research area (keywords): comparative pharmacology, pre-clinical research, translational sciences	
Research skills (keywords): cell culture, drug testing, confocal imaging, mitochondrial functions profiling, high-content cell imaging, complex data analysis, 3Rs, zebrafish	
Faculty/School/Department: Institute of Pharmaceutical Sciences/AEFS	
Location: FWB Waterloo; Guy's Campus; Denmark Hill Campus	

Project description:

Metformin is the most prescribed glucose-lowering therapy for the management of type 2 diabetes and is used daily by over 200 million patients worldwide. Its antidiabetic effects of are mediated through the modulation of mitochondrial functions and the inhibition of hepatic gluconeogenesis. However, emerging evidence suggests that metformin may also induce additional therapeutic benefits, including anti-inflammatory, immunomodulatory, anti-cancer, and anti-ageing effects (Foretz et al., 2023). Non-mammalian pre-clinical models – such as zebrafish, C. elegans, and Drosophila - have played a key role in unravelling such secondary effects (Onken et al., 2022; de Olivera et al, 2019). However, it is currently unclear to what extent the effects observed in those species can be translated to humans. This is largely due to the lack of quantitative understanding of the similarity of relevant inter-species responses (e.g. AMPK-mediated metabolic responses).

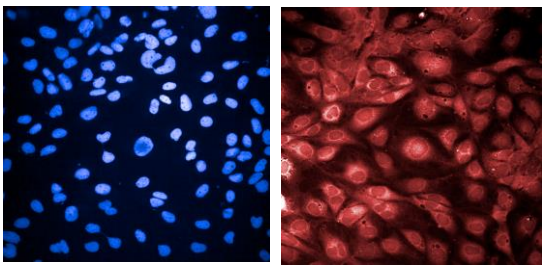


Figure 1. *Zebrafish liver cells stained with Hoechst 33342 and Mitochondrial Deep Red to visualise, respectively, nucleus (left image) and mitochondria (right image) (credit: Margiotta-Casaluci Lab).*

In this project, the student will use high-content methodologies to investigate the similarity between metabolic responses induced by metformin in human and zebrafish liver cells. Drug-mediated effects on mitochondrial energetics will be characterised using the SeaHorse XF technology, which allows to measure key mitochondrial health parameters, such as oxygen consumption rate, proton efflux rate, extracellular acidification rate, and ATP production rates, in real time in live cells. Together, these measurements will provide a systems-level view of cellular metabolic function in cells exposed to the drug. This data will be integrated with morphological data acquired via high-content imaging to quantify the effect of the drug on relevant cell phenotypes, such as mitochondria distribution and nucleus morphology. Throughout the project, the student will benefit from working in close collaboration with both Dr Luigi Margiotta-Casaluci (expert in comparative pharmacology, drug safety, & zebrafish biology) and Dr Anna Zoccarato (expert in mitochondrial functions and metabolic medicine), and from being exposed to a diverse set of expertise and advanced experimental techniques.

References: 1) Foretz et al. (2023) DOI: https://doi.org/10.1038/s41574-023-00833-4; **2)** Onken et al. (2022) DOI: 10.1111/acel.13488; **3)** de Olivera et al. (2019) DOI: 10.1016/j.jhep.2018.11.034

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -37-

Supervisor(s): Setsuko Sahara	**E-mail (lead supervisor):** setsuko.sahara@kcl.ac.uk

Project title: Modelling the brain size disorder in the dish

Research area (keywords): Stem cells, Brain size, Cortical development, Neurogenesis and Gliogenesis

Research skills (keywords): human and mouse ES/iPS cell culture**,** Molecular biology, FISH, Bioinformatics, CRISPR editing, imaging and analysis

Faculty/School/Department: Centre for Developmental Neurobiology

Location: CDN functional genomics centre, New Hunt House and Hodgkin Building, Guys Campus

Malan syndrome is a rare genetic overgrowth disorder, clinically characterised by unusual facial features and macrocephaly associated with intellectual disability. Most reported cases are sporadic and caused by de novo mutations in a single transcription factor, Nfix. The overall aim of this project is to identify the developmental mechanisms underlying Malan syndrome macrocephaly by focusing on Nfix isoform expression and function.

Through transcriptome analysis of the embryonic cortex where the onset of neurogenesis is delayed, we identified Nuclear Factor I (NFI) transcription factors (TFs) as potential candidates in maintaining expansive neuroepithelial (NE) fates. Concomitantly, through gain-of-function (GOF) experiments in ES cell-derived cortical progenitor differentiation and in vivo GOF studies in the developing mouse cortex, we found isoform-specific roles for a member of the NFI TFs, Nfix, in radial glial (RG) differentiation. A shorter isoform exhibited robust RG induction activity, while a longer isoform suppressed it. Here, we hypothesise that Nfix isoforms, generated through alternative transcriptional start sites, may balance the expansive and neurogenic potentials of progenitors, thereby playing a crucial role during brain development.

To gain mechanistic insights into Malan syndrome brain overgrowth, we will generate several mutant lines and analyse the impact of the disease-causing mutations on Nfix isoform expression and function in ES cell-based mouse and human cortical differentiation models. Throughout the project, the student will learn stem cell manipulation, culturing, and various molecular and gene-editing techniques. This project is well-suited for students interested in stem cell fate decisions, sex-biased gene expression, CRISPR-based technologies, and culturing mouse ES and human iPS cells. Whilst prior experience and knowledge in cell culture and molecular biology are not essential, they are preferable.

References:

Sahara, S. & O'Leary, D. D. M. Fgf10 Regulates Transition Period of Cortical Stem Cell Differentiation to Radial Glia Controlling Generation of Neurons and Basal Progenitors. *Neuron* **63**, 48–62 (2009).

Priolo, M. *et al.* Further delineation of Malan syndrome. *Human Mutation* **39**, 1226–1237 (2018).

Gaspard, N. *et al.* Generation of cortical neurons from mouse embryonic stem cells. *Nat. Protocols* **4**, 1454–1463 (2009).

Lancaster, M. A. *et al.* Cerebral organoids model human brain development and microcephaly. *Nature* **501**, 373–379 (2013).

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MSc/MRes Biomedical & Molecular Sciences Research

Research Project -38-

Supervisor(s): Francesca M. Spagnoli	**E-mail (lead supervisor):** francesca.spagnoli@kcl.ac.uk
Project title: Regulation of cellular plasticity in the hepato-pancreato-biliary organ system	
Research area (keywords): stem cell, development, regenerative medicine, liver, pancreas, diabetes	
Research skills (keywords): sc-RNASeq analysis, FACS-Sorting, immunofluorescence, confocal imaging, image analyses, gene expression analysis, cell culture	
Faculty/School/Department: Faculty of Life Sciences & Medicine/ School of Basic and Medical Biosciences / Centre for Gene Therapy and Regenerative Medicine	
Location: Guy's Campus	

Project description:

Cellular plasticity, the ability of a cell to adopt alternative fates or acquire new identities, is relevant to diverse fields, including developmental and stem cell biology, regenerative medicine. Insights into these mechanisms could provide us with tools for enhancing regeneration, but also shed light on organogenesis and define novel cell therapies. Previous investigations in the Spagnoli group have focused on the lineage plasticity between liver and pancreas with the aim to generate insulin-producing pancreatic beta-cells starting from the liver towards a cell replacement therapy for diabetes. **More recently, our work has underscored that sustained plasticity is also a key feature of hepato-pancreato-biliary (HPB) development** [Willnow et al. *Nature* 2021]. We discovered a subpopulation of progenitors, which resides in a specialized *niche* in a plastic/multipotent state and contributes cells not only to the pancreas but also to the liver for an extended window of time, beyond individual lineage fate specification in the mouse. Further understanding of such population during development will elucidate programs underlying growth and regeneration of these related organs. Moreover, investigations are required to determine whether a subset of cells with similar plasticity resides in the adult HPB tissues. *This project builds on these findings and will investigate the properties of this newly identified progenitor population, called intermediate progenitors (IMP), in the adult liver and extra-hepatic tissues.*

We aim to answer fundamental outstanding questions about the newly discovered IMP cell subtype, including its location and persistence in adult tissues, using lineage tracing experiments combined with confocal and light-sheet microcopy. We also aim at completing the characterization of the molecular and functional properties of the newly IMP cells and establish a culture system to recapitulate the stem cells-derived IMP *niche* in a dish.

References:

Cozzitorto C, Mueller L, Ruzittu S, Mah N, Willnow D, Darrigrand JF, Wilson H, Khosravinia D, Mahmoud AA, Risolino M, Selleri L, Spagnoli FM. A Specialized Niche in the Pancreatic Microenvironment Promotes Endocrine Differentiation. Dev Cell. 2020 55:150-162.e6. doi: 10.1016/j.devcel.2020.08.003.

Willnow D, Benary U, Margineanu A, Vignola ML, Konrath F, Pongrac IM, Karimaddini Z, Vigilante A, Wolf J, Spagnoli FM. Quantitative lineage analysis identifies a hepato-pancreato-biliary progenitor niche. *Nature*. 2021 597, pages 87–91 (2021). doi: 10.1038/s41586-021-03844-1

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -39-

Supervisor(s): Dr Sally Kim & Dr Simon Pitchford	**E-mail (lead supervisor):** sally.kim@kcl.ac.uk

Project title: Deciphering the pro-repair capacity of alveolar epithelial cell-derived extracellular vesicles in lung injury

Research area (keywords): lung tissue repair, extracellular vesicles, respiratory medicine

Research skills (keywords): cell culture, ex-vivo tissue culture, immunostaining, fluorescence imaging, molecular biology

Faculty/School/Department: FoSLM / Cancer & Pharmaceutical Science / Institute of Pharmaceutical Science

Location: Franklin-Wilkins Building, Waterloo Campus

Project description:

Chronic lung infections (e.g. long Covid-19) or lung trauma lead to lung damage that is not currently treatable. Extracellular vesicles (EVs) are nanosized particles (50–1000 nm) that transfer biological information (signalling proteins, nucleic acids and lipids) between cells [1]. Many studies that investigate the pro-repair effects of EVs to treat lung damage utilise mesenchymal stem/stromal cell (MSC)-derived EVs, mainly for their anti-inflammatory effects [2]. Alveolar type 2 cells are alveolar epithelial progenitor cells that can repopulate the injured alveolar epithelium through self-renewal and differentiation to alveolar type 1 cells that provide structure to alveoli [3]. Since it is well established that stem/progenitor cells drive tissue repair through the release of EVs [4], alveolar epithelial cell-derived EVs warrant investigation for their potential pro-repair effects.

To determine the pro-repair effects of alveolar epithelial cell-derived EVs, a state-of-art ex-vivo lung tissue slices will be generated from mouse lungs after LPS-injury. These 300um thick lung slices are essentially 'mini-lungs' that contain most cell types present in the lung in their natural orientation and have been demonstrated as an excellent model to study lung injury and repair [5].

Hypothesis: Alveolar epithelial cell-derived EVs have pro-repair effects when applied *ex-vivo* to lipopolysaccharide (LPS)-injured lung slices.

Aim 1. Isolate primary alveolar epithelial cells from mouse lungs by using magnetic bead separation using an established protocol [6].

Aim 2. Isolate and characterise alveolar epithelial cell-derived EVs by using electron microscopy and nanoparticle tracking analysis (NanoSight).

Aim 3. Assess pro-repair effects of EVs (from Aim 2) using lung tissue slices generated from LPS-injured mouse lungs. Determine the level of injury/repair by histology and immunostaining (e.g. Podoplanin and proSP-C for alveolar type 1 and 2 cells, Ki67 for proliferating cells).

Successful completion of this project will identify the extent of pro-repair effects of alveolar epithelial cell-derived EVs for the first time and investigate potential use in repairing lung injury.

References: [1] Bjørge and Kim *et al. Biomater Sci.* 2018; 6(1):60. [2] Ma *et al. Front Bioeng Biotechnol.* 2022; 10:845042. [3] Zacharias *et al. Nature* 2018; 555(7695):251. [4] Kadota *et al. Eur Respir Rev.* 2022; 31(163):210106. [5] Kim and Mongey *et al. Biomaterials* 2021; 267:120480. [6] Cheong *et al. Front. Cell Dev Biol.* 8:577201.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -40-

Supervisor(s): Dr Sally Kim & Dr Driton Vllasaliu	**E-mail (lead supervisor):** sally.kim@kcl.ac.uk

Project title: How can alveolar epithelial cells be stimulated in-vitro to have more pro-repair effects?

Research area (keywords): lung tissue repair, extracellular vesicles, respiratory medicine

Research skills (keywords): cell culture, ex-vivo tissue culture, immunostaining, fluorescence imaging, molecular biology

Faculty/School/Department: FoSLM / Cancer & Pharmaceutical Science / Institute of Pharmaceutical Science

Location: Franklin-Wilkins Building, Waterloo Campus

Project description:

Alveolar type 2 (AT2) cells are progenitor cells in the alveolar epithelium that are able to repopulate the alveolar space through self-renewal and differentiation to alveolar type 1 cells that provide structure to alveoli [1]. AT2 cells have shown similar therapeutic effects to the well-known mesenchymal stem/stromal cells (MSCs) in reducing acute lung injury [2]. It is well established that stem/progenitor cells drive tissue repair through the release of extracellular vesicles (EVs) [3]. EVs are heterogeneous nanoscale messengers (50–1000 nm) that transfer biological information between cells through encapsulated signalling proteins, nucleic acids and lipids [4].

EVs can be pre-conditioned to yield desirable properties and improve their potential therapeutic effects. For instance, EVs released by cells under stressful conditions have shown to express molecules such as CD47 (do not eat me signals) that prevent their uptake by phagocytic cells, delaying EV clearance from circulation and thereby increasing their effects [5].

Hypothesis: Alveolar epithelial cells can be stimulated in-vitro to yield EVs that have pro-migratory, wound healing effects on human lung epithelial cells.

The aims of the project are:

1) Isolate primary alveolar epithelial cells from mouse lungs by using magnetic bead separation using an established protocol [6].
2) Compare the effects of pre-conditioning alveolar epithelial cells to enhance EV secretion (e.g. exposing to hypoxic conditions, treating with EVs collected from lipopolysaccharide-injured cells) by measuring size and concentration of EVs.
3) Determine potential pro-repair effects of alveolar epithelial cell-derived EVs using scratch wound assays with A549 and BEAS-2B epithelial cell lines.

The outcomes of this innovative, proof-of-principle project will determine optimal conditions in which alveolar epithelial cells can generate enhanced EV secretion with pro-migratory effects.

References:

[1] Zacharias *et al. Nature* 2018; 555(7695):251. [2] Guillamat-Prats *et al. Cells* 2020; 9(8):1816. [3] Kadota *et al. Eur Respir Rev.* 2022; 31(163):210106. [4] Bjørge and Kim *et al. Biomater Sci.* 2018; 6(1):60. [5] Edelmann MJ, Kima PE. *Zoonoses (Burlingt)* 2022;2:14. [6] Cheong S-S *et al. Front. Cell Dev Biol.* 8:577201.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -41-

Supervisor(s):	**E-mail (lead supervisor):**
Dr Antonio Riva Miss Phoebe Tsou	a.riva@researchinliver.org.uk

Project title: *Sex-specific immune differences in primary biliary cholangitis*

Research area (keywords): liver disease, autoimmunity, sex-based differences, immunology

Research skills (keywords): handling of human plasma/serum samples, uniplex/multiplex quantification assays, biostatistics

Faculty/School/Department: The Roger Williams Institute of Hepatology, affiliated with the Faculty of Life Sciences and Medicine / School of Immunology and Microbial Sciences / Department of Inflammation Biology

Location: The Roger Williams Institute of Hepatology, Denmark Hill, 111 Coldharbour Lane, London SE5 9NT

Project description:

Background and aim. Immune responses are naturally stronger in females than males, which is advantageous against infection and disease. However, females are also more predisposed to autoimmunity. Primary biliary cholangitis (PBC), a very common autoimmune liver disease, is indeed around ten times more likely to occur in post-menopausal women than in men, despite considerable geographical variation. PBC is a severely debilitating condition, which leads to progressive liver damage, cirrhosis and liver failure, and it is a major cause of liver-related mortality in the UK. Current treatments are not curative and only improve symptoms in a small proportion of patients, often carrying non-negligible side effects; even liver transplantation cannot halt disease progression but only delays it.

It has been reported that while male PBC patients may initially present with fewer symptoms, which often leads to delayed or missed diagnosis, they may be more susceptible to a faster and more severe course, with greater risk of disease progression and death. Thus, besides occurring at different rates, this disease may also take different trajectories in the two sexes.

How sex-related immune differences contribute to PBC is currently unclear. We have previously identified distinct immunological features associated with disease development, progression and – potentially – treatment response in female PBC patients; however, these profiles have never been investigated in male PBC patients, and this is the aim of this project.

We hope that these results will help enhance our understanding of how sex immune differences contribute to PBC, with the possibility to identify novel immunotherapeutic targets for this debilitating disease.

Technical aspects. The project will involve technologies such as high-sensitivity multiplex ELISAs, Luminex, and potentially flow cytometry in a fully equipped state-of-the-art laboratory at the Roger Williams Institute of Hepatology, a purpose-built internationally renowned research centre entirely dedicated to translational research in liver disease. Samples, instrumentation and a solid technical and statistical support are available at the Institute. The candidate will also be involved in data analysis, which will be a stimulating challenge and help develop detailed problem-solving skills and critical thinking.

References: Riva A. Front Immunol 14:1178541 (2023). Lotersztajn S, Riva A, et al. Z Gastroenterol 60, 58-66 (2022). Riva A, et al. Front Physiol 12:632502 (2021). Sharma L, Riva A. Microorganisms 8(11):1744 (2020). Riva A and Gray EH, et al. JHEP Rep 2, 100151 (2020). Riva A, et al. Gut 67, 918-930 (2018). Riva A, Chokshi S. Hepatol Internat 12(3):223-236 (2018). Markwick LJ, Riva A, et al. Gastroenterology 148, 590-602.e510 (2015).

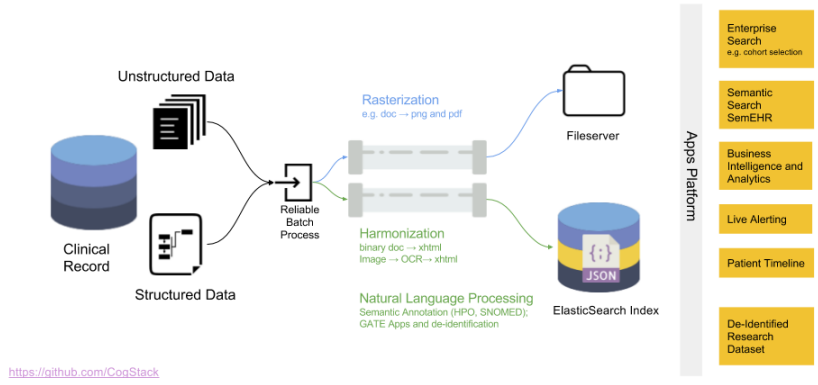
MSc/MRes Biomedical & Molecular Sciences Research

Research Project -42-

Supervisor(s): Dr Antonio de Marvao; Maternal Cardiovascular Medicine & Genomics	**E-mail (lead supervisor):** antonio.de_marvao@kcl.ac.uk
Project title: Language AI for the automated identification, genetic testing and risk stratification of patients with inherited cardiac conditions	
Research area (keywords): AI; genomics; cardiology; inherited cardiac conditions; natural language processing (NLP); large scale language models; health informatics; CogStack	
Research skills (keywords): machine learning; bioinformatics; statistics	
Faculty/School/Department: Department of Women and Children's Health & School of Cardiovascular and Metabolic Medicine and Sciences King's College London	
Location: Denmark Hill and / or St Thomas' Hospital campus	

Background: Highly detailed longitudinal characterisation of patient phenotypes has an essential role in clinical and genomic research. However, phenotypic descriptions and longterm outcome data, are often stored in the form of unstructured clinical data, such as in hospital letters to GPs, that is not amenable to manual inspection and collation at scale. CogStack (https://cogstack.org/) is an open-source platform developed within KCL, that is able to index free text electronic patient records. This enables the application of natural language processing (NLP) and machine learning (ML) techniques to analyse and annotate large amounts of unstructured clinical data in standardised clinical terminologies (SnomedCT/UMLS). The Genomic AI Network of Excellence (GAIN), is a programme funded by NHS England and aims to enhance AI use in Genomic Medicine.

Project: The aim of this GAIN project is to automatically identify patients with Inherited Cardiac Conditions by using an AI algorithm to scan Electronic Health Records. This includes longitudinal patient demographics, investigations, as well as procedures and clinical diagnoses. Identified patients will be invited for genetic testing and specialised cardiac care. The student will help test, validate, and implement NLP solutions for analysing unstructured clinical data (over 500 million diagnostic results, reports and documents). The student will then work on predictive modelling and disease forecasting. During this project they will develop quantitative skills, such as classical statistics, large-scale data handling, scripting, and open-source software usage.



References: 1 - Jackson, R. et al. CogStack - experiences of deploying integrated information retrieval and extraction services in the NHS. BMC. 2018. https://doi.org/10.1186/s12911-018-0623-9

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -43-

Supervisor(s): Sandrine Thuret Co-supervisor: Sahand Farmand	**E-mail (lead supervisor):** sandrine.1.thuret@kcl.ac.uk

Project title: Investigating the Role of Clusterin in Human Hippocampal Neurogenesis: an In Vitro Approach

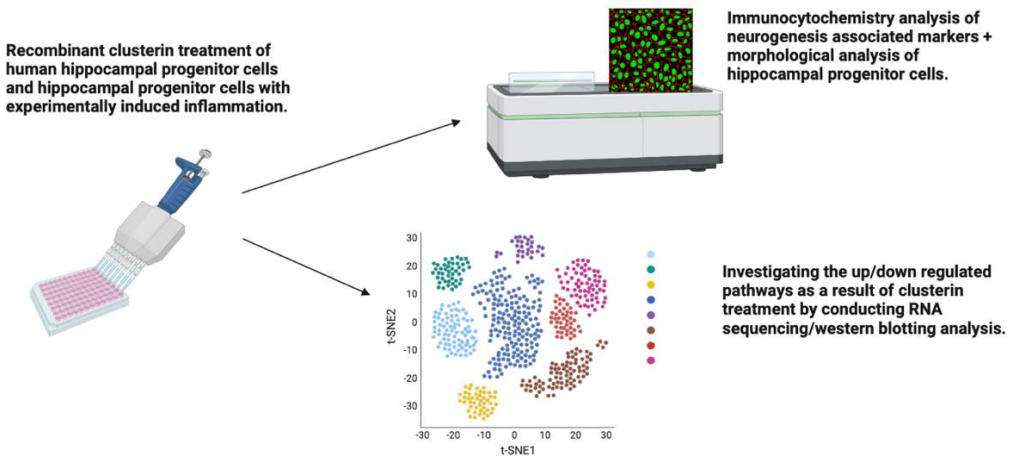
Research area (keywords): Hippocampal Neurogenesis, Stem cell, Ageing, Clusterin, Exercise.

Research skills (keywords): Stem cell culture, Immunocytochemistry, high content imaging.

Faculty/School/Department: Basic and Clinical Neuroscience, IoPPN.

Location: Maurice Wohl Clinical Neuroscience Inst, KCL Denmark hill campus, London, SE5 9RX.

Project description: Adult hippocampal neurogenesis (AHN), often described as generation of new neurons from the neural stem cells (NSCs) in the dentate gyrus of the hippocampus[1] has shown to be a key element in regulating different aspects of cognition such a mood and spatial memory[2]. Rodent studies have demonstrated that AHN is sensitive to life-style interventions such as exercise[2]. Specifically, exercise has shown to improve the neurogenic process which in turn improves certain cognitive abilities, such as the ability to discriminate between non-identical but overlapping patterns[2]. While the pathways through which exercise regulates neurogenesis are yet to be identified, several rodent studies have demonstrated that blood factors could potentially be the key underlying mediators of these effects[3]. Recently, a mice study demonstrated that injection of plasma collected from a running mice (running plasma), has the ability to lower inflammation in a sedentary mice model[4]. In the same study, running plasma was shown to have significantly higher concentrations of clusterin (CLU), a complement cascade inhibitor, compared to the plasma collected from sedentary mice. Notably, systematic injection of plasma, was shown to reduce hippocampal inflammation[4], raising the question of clusterin's role in hippocampal neurogenesis. This project is an opportunity to close this knowledge gap and here we propose to investigate the effect of clusterin, in an in vitro model of hippocampal neurogenesis well established in the Thuret Lab[5] by investigating the effect of clusterin on the fate of human hippocampal stem cells. To do this, the student will be trained to perform stem cell culture, use immunostaining assays to measure and investigate expression of neurogenesis associated markers and perform high content imaging analysis. Additionally, the student will have the opportunity to expand their data and statistical analysis skills.



References: [1] Moreno-Jiménez, E. P., Terreros-Roncal, J., Flor-García, M., Rábano, A., & Llorens-Martín, M. (2021). Evidences for Adult Hippocampal Neurogenesis in Humans. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *41*(12), 2541–2553. https://doi.org/10.1523/JNEUROSCI.0675-20.2020. **[2]** van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(23), 13427–13431. https://doi.org/10.1073/pnas.96.23.13g427. **[3]** Horowitz, A. M., Fan, X., Bieri, G., Smith, L. K., Sanchez-Diaz, C. I., Schroer, A. B., Gontier, G., Casaletto, K. B., Kramer, J. H., Williams, K. E., & Villeda, S. A. (2020). Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. *Science (New York, N.Y.)*, *369*(6500), 167–173. https://doi.org/10.1126/science.aaw2622. **[4]** De Miguel, Z., Khoury, N., Betley, M. J., Lehallier, B., Willoughby, D., Olsson, N., Yang, A. C., Hahn, O., Lu, N., Vest, R. T., Bonanno, L. N., Yerra, L., Zhang, L., Saw, N. L., Fairchild, J. K., Lee, D., Zhang, H., McAlpine, P. L., Contrepois, K., Shamloo, M., … Wyss-Coray, T. (2021). Exercise plasma boosts memory and dampens brain inflammation via clusterin. *Nature*, *600*(7889), 494–499. https://doi.org/10.1038/s41586-021-04183-x. **[5]** The serum metabolome mediates the concert of diet, exercise, and neurogenesis, determining the risk for cognitive decline and dementia. Du Preez A…Thuret S. (2022). Alzheimers Dement.;18(4):654-675. doi: 10.1002/alz.12428. See further references at https://www.thuretlab.com/

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -44-

Supervisor(s): Ramin Raouf	**E-mail (lead supervisor):** ramin.raouf@kcl.ac.uk

Project title: Exploring Headache Mechanisms through an Innovative Organ-on-a-Chip Culture System

Research area (keywords): Neuroscience, Pain, Organ-on-a-chip,

Research skills (keywords): cell culture, calcium imaging, electrophysiology, microfluidic cultures

Faculty/School/Department: IoPPN/Wolfson SPaRC

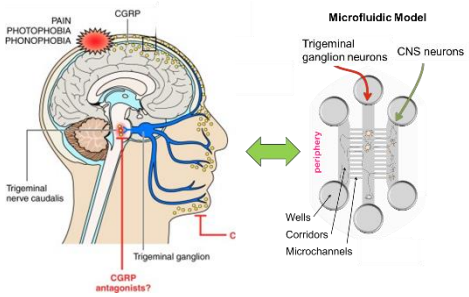
Location: King's College London, Guy's Campus, Wolfson Sensory, Pain and Regeneration Centre

Project description:

In this project we will investigate the enigma of migraine pain. This project aims to address the molecular mechanisms of migraine headaches, a pressing clinical challenge. By focusing on the intricate network of trigeminal ganglion (TG) neurons and their synaptic connections responsible for transmitting headache pain signals, the study aims to unravel the mechanisms behind the effects of mediators which are intricately linked to migraine. The breakthrough potential of this investigation lies in deciphering molecular mechanism of the changes that ultimately lead to headache pain as a starting point for developing novel therapeutics. Leveraging cutting-edge organ-on-a-chip microfluidic cultures and cell imaging techniques the project will focus on pathological changes in trigeminal neurons and their synaptic connections that could contribute to headache pain.

Several neurotransmitters and neuropeptides, such as calcitonin gene related peptide (CGRP), that are produced in the trigeminal ganglion neurons are released during migraine headache. The success of the anti CGRP monoclonal antibodies in migraine treatment underscores the importance of CGRP in migraine headache. Increasing evidence suggests that CGRP plays an important role in sensitization of TG neurons and enhanced sensation of pain, and that this sensitization of the TG neurons contributes to headache pain. The mechanisms of actions of CGRP leading to sensitization of TG neurons, and particularly sensitization of the axons of these neurons, where the transduction and transmission of pain signals occur, remain largely unknown.

This project aims to determine the mechanism of sensitization of TG neurons and changes in synaptic transmission by calcitonin gene related peptide (CGRP), that is released during migraine headache. Using a novel microfluidic-based cell culture platform developed in Raouf lab, you will culture trigeminal neurons in microfluidic devices and investigate the mechanisms of CGRP-mediated changes in activation of axons using a number of cutting-edge techniques.



References:

Tsantoulas, C. et al., (2013). Probing Functional Properties of Nociceptive Axons Using a Microfluidic Culture System. PLoS One 8(11), e80722. Vysokov N. et al., The role of NaV channels in synaptic transmission after axotomy in a microfluidic culture platform. Sci Rep. 2019 Sep 9;9(1):12915. **Ho, T.W et al., (2010). CGRP and its receptors provide new insights into migraine pathophysiology. Nat Rev Neurol 6(10), 573-582.** Haanes KA, Edvinsson L. Pathophysiological Mechanisms in Migraine and the Identification of New Therapeutic Targets. CNS Drugs. 2019 Jun;33(6):525-537.

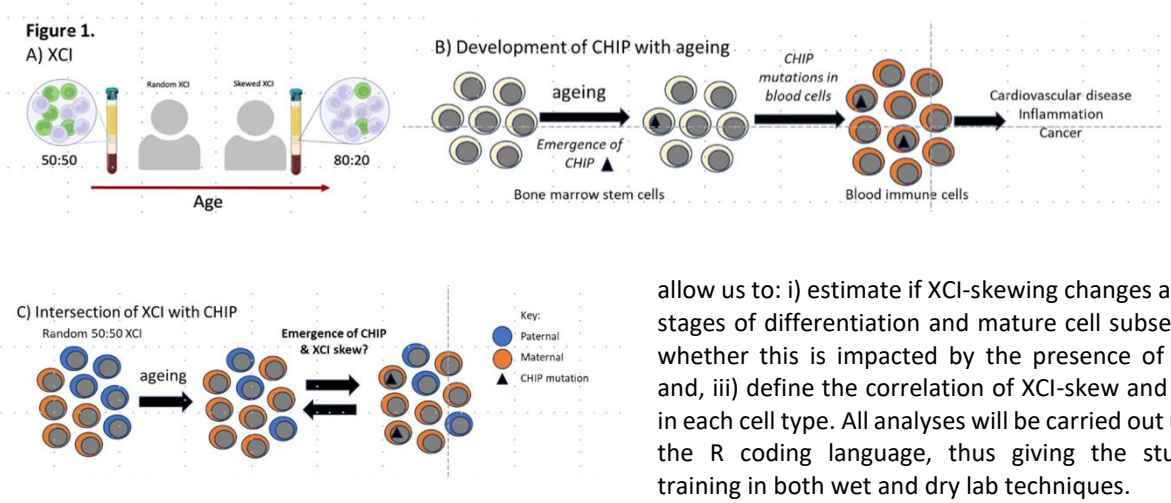
MSc/MRes Biomedical & Molecular Sciences Research

Research Project -45-

Supervisor(s): Dr Lynn Quek, Prof. Kerrin Small, and Dr Amy Roberts	**E-mail (lead supervisor):** lynn.quek@kcl.ac.uk
Project title: Understanding the clonal expansion of blood cells using two molecular phenotypes	
Research area (keywords): Ageing; molecular biology; somatic mutations	
Research skills (keywords): PCR; gene fragment analysis; digital droplet PCR genotyping; R coding	
Faculty/School/Department: Comprehensive Cancer Centre and Department of Twin Research & Genetic Epidemiology	
Location: Denmark Hill Campus and St Thomas' Hospital Campus	

Background: Clonal expansion of blood cells, whereby increasing numbers of immune cells derive from a common stem cell progenitor, is a common age acquired cellular phenotype. This is associated with adverse health outcomes, including cancer and atherosclerotic cardiovascular disease (1). However, how clonal expansion affects disease risk is not fully understood. In females, clonal expansion can be measured by two discrete molecular markers: i) changes to X Chromosome Inactivation (XCI) ratios and ii) the identification of somatic mutations. XCI is the mechanism which equalises the gene dosage between the X and Y chromosomes through the functional silencing of one X chromosome in female XX cells. Therefore, each female cell has either an active maternal or paternal X, and because the selection is random in humans, there is an expected 50:50 ratio across a tissue. However, significant deviation from this ratio, termed XCI-skew, is commonly observed in blood tissue of ageing females and is thought to reflect changes to the underlying haematopoietic stem cells (ref 2; **Fig.1A**). Likewise, identification of somatic mutations which are shared across ≥4% of blood cells, termed clonal haematopoiesis of indeterminate potential (CHIP), reflects expansion of haematopoietic stem cells with the mutation (ref 3; **Fig. 1B**). Whether immune cells harbouring the CHIP mutations lead to XCI-skew has yet to be defined (**Fig. 1C**), and studying the concurrence of XCI and CHIP will increase our understanding of disease risk.

Aims and Objectives: The aim of the project is to define whether XCI-skew and CHIP co-occur in isolated immune cell subsets. To do this, we will use DNA from haematopoietic precursors from bone marrow, and mature immune cells from blood samples, from 100 female volunteers (average age =68 years). We will measure XCI and CHIP using HUMARA and digital droplet (dd)PCR genotyping, respectively. These data will allow us to: i) estimate if XCI-skewing changes across stages of differentiation and mature cell subsets, ii) whether this is impacted by the presence of CHIP and, iii) define the correlation of XCI-skew and CHIP in each cell type. All analyses will be carried out using the R coding language, thus giving the student training in both wet and dry lab techniques.



References: 1) Jaiswal, et al. *N Engl J Med* 2014; 2) Roberts, et al. *eLife*. 2022; 2) Zheng X, et al. *Blood* 2023

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -46-

Supervisor(s): Prof. Kerrin Small and Dr Amy Roberts	**E-mail (lead supervisor):** kerrin.small@kcl.ac.uk

Project title: Identifying the risk factors for skewed X chromosome inactivation: a longitudinal study

Research area (keywords): epidemiology; ageing; molecular biology

Research skills (keywords): PCR; gene fragment analysis; R coding; heritability analysis; regression

Faculty/School/Department: Department of Twin Research & Genetic Epidemiology

Location: St Thomas' Hospital Campus

Background: X chromosome inactivation (XCI) is the mammalian process which equalises the gene dosage between the X and Y chromosomes. The choice of which X is silenced is random and occurs early in development, resulting in an expected 50:50 ratio across tissues in females. However, some females display a "skewed" pattern of XCI in which >80% of cells have the same X silenced (**Fig.1**). This is termed XCI-skew, and its prevalence increases with age in human immune cells, with over 30% of females over 60 years old displaying this cellular phenotype. Our cross-sectional study using the TwinsUK population cohort (n=1,575) demonstrated that XCI-skew is associated with adverse health outcomes in humans. Specifically, XCI-skew correlates with a clinical score of cardiovascular risk and is predictive of future cancer diagnosis in a 10-year follow-up (**1**). Though heritability analyses show both genetic and environmental factors influence risk of XCI-skew (**2**), we have not yet identified the specific risk factors. Fascinatingly, we have identified identical twins who have aged discordantly for XCI (**Fig.2**). Studying XCI longitudinally will help us better understand its causes and consequences.

Aims and objectives: The aim of the project is to identifying risk factors which affect changes to XCI across time. To do this, we will measure XCI in DNA samples from 200 female volunteers from the TwinsUK cohort at two time points (median age time point 1=55; median years between time points=15), using the PCR-based Human Androgen Receptor Assay (HUMARA). We will define the longitudinal trajectories of XCI, identify discordant twins, and calculate the heritability of the rate of change. Finally, we will match the XCI data with the phenotypic data in TwinsUK to assess the impact of environmental risk factors (e.g. smoking) and age-related phenotypes (e.g. menopause).

All analyses will be carried out using the R coding language, thus giving the student training in both wet and dry lab techniques.

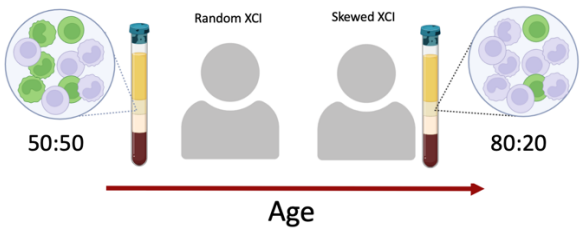


Figure 1: schematic diagram of XCI-skew.

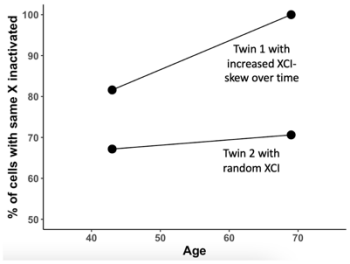


Figure 2: Identical twins discordant for XCI

References: 1) Roberts, et al. *eLife*, 2022; **2)** Zito, et al. Nature Communications, 2019

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -47-

Supervisor(s): Filippo Prischi, Miraz Rahman	**E-mail (lead supervisor):** filippo.prischi@kcl.ac.uk; miraz.rahman@kcl.ac.uk
Project title: Design and Development of novel RNA binding protein inhibitors	
Research area (keywords): Biochemistry, Medicinal Chemistry, Computational Biology	
Research skills (keywords): Biochemistry & Medicinal Chemistry, Protein expression and purification, biophysical techniques, Chemical synthesis, structural biology.	
Faculty/School/Department: FoLSM/Randall Centre for Cell and Molecular Biophysics/Institute of Pharmaceutical Science	
Location: Guy's Campus/Franklin-Wilkins Building	

Project description: Background. The human heterogeneous nuclear ribonucleoprotein (hnRNP) A1 is a multifunctional RNA-binding protein with a key role in RNA metabolism, and deregulation of its functions has been linked to neurodegenerative diseases, viral gene expression, tumour aggressiveness and chemoresistance. This has recently fuelled efforts to develop novel therapeutics that modulates its RNA binding activities. We have recently shown that hnRNPA1 N-terminal RNA binding domain (UP1) contains several pockets capable of binding small molecules. We carried out a large fragment screening on UP1 protein crystals and identified 36 hits. Our high-resolution structures provide rapid routes for the rational development of a range of different potent inhibitors and chemical tools for studying molecular mechanisms of hnRNPA1 mediated splicing regulation.

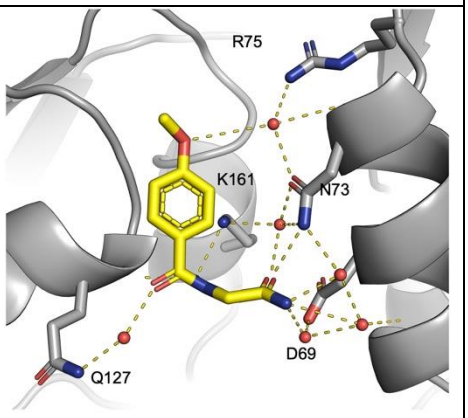


Fig. 1. Fragment bound to UP1 (PDB ID: 9F4T)

Project aims. In this project we aim to design and experimentally validate compounds able to inhibit hnRNPA1. Follow-up compounds will be rationally designed via (i) fragment merging and linking, which will be directly inferred from available structures, and/or (ii) using computational tools (AI-guided design and/or MD simulations). Compounds will be screened *in silico,* and the best predicted binders will be synthetised after necessary optimisation to improve affinity and drug like properties. We will express and purify UP1 and carry out affinity binding measurements and RNA-binding inhibition assays using biophysical techniques (ITC/MST). Compounds with high binding affinity and ability to interfere with RNA/DNA binding will be tested on lung cancer cell for their ability to overcome chemoresistance. The crystal structure of UP1 in complex with these compounds will provide key structure–function relationships information. Techniques. The project is highly interdisciplinary and will involve a wide range of computational and experimental techniques, including compounds design and chemical synthesis, compound purification using prep-HPLC, characterisation using NMR and mass spec techniques, cell culture, protein expression and purification, X-Ray crystallography, and biophysical assays (including ITC/MST).

References: (1) Roucairol et al. J. Chem. Inf. Model. (2024); (2) Roy *et al. Nucleic Acids Res* (2014)

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -48-

Supervisor(s): **Dr Konstantin Roeder**	**E-mail (lead supervisor):** Konstantin.roeder@kcl.ac.uk

Project title: Investigation of Structural Dynamics in a Neomycin-sensing Riboswitch

Research area (keywords): Computational structural biology, RNA structural dynamics, Ligand binding

Research skills (keywords): Biomolecular Simulations, RNA structure prediction

Faculty/School/Department: FoLSM/BMBS/Randall Centre for Cell and Molecular Biophysics

Location: New Hunt's House, Guy's Campus

Project description: Background: Functional RNAs are remarkable different from their protein counterparts. Instead of a well-defined three-dimensional structure that is linked to function, RNAs exhibit what is termed structural dynamics and structural polymorphism. There are multiple competing structures, and their dynamic interplay defines the overall behaviour of an RNA molecule.

Advanced computational methods are well-placed to study such RNAs, and extract a global view of their structure. Often, experimental findings indicate RNAs of interest, that can then be studied in detail with simulations. Computational findings can then be revalidated by experiment.

One emerging area of interest is the use of RNAs as drug targets. While they are functionally important and have been implicated in almost any major disease, few drugs target RNAs. Our current lack of structural understanding of these RNAs is a main contributor to this under-utilisation.

Project outline: In this project, we will consider one RNA that is known to interact with small molecules – a neomycin-sensing riboswitch. Riboswitches are mostly found in the 5'-untranslated regions of bacterial RNA and regulate gene expression via binding to small molecules. The riboswitch under consideration here binds to neomycin. This switching is highly sensitive to the ligand (1). The actual binding an occur at different binding sites, but structural changes as a result seem to be similar (2). In addition, the binding process involves multiple RNA structures (3).

We will use state-of-the-art computational methods to explore the energy landscape of the riboswitch in absence and presence of the small molecule ligands. This will reveal the structural ensemble and dynamics of this RNA. In other RNAs, we have observed a rebalancing of the RNA structural ensemble, rather than the emergence of novel states upon binding. Our working hypothesis is that this is a feature of functional RNAs and will enable drug targeting. This project might provide further evidence for this idea.

Techniques: The project is purely computational, and will use a variety of methods to study biomolecules with physics-based simulations.

References:

(1) Duchardt-Ferner et al. Angew. Chem. (2015) DOI: 10.1002/anie.201507365
(2) Heel and Breuker ChemPlusChem (2024) DOI: 10.1002/cplu.202400178
(3) Overbeck et al. Angew. Chem. (2023) DOI: 10.1002/anie.202218064

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -49-

Supervisor(s): Matt Grubb	**E-mail (lead supervisor):** matthew.grubb@kcl.ac.uk

Project title: The cellular basis of persistent COVID-19-related anosmia

Research area (keywords): Neuroscience, Sensory biology, COVID-19

Research skills (keywords): Histology, human tissue, immunohistochemistry, confocal microscopy, quantitative image analysis

Faculty/School/Department: IoPPN/Neuroscience/Centre for Developmental Neurobiology

Location: New Hunt's House, Guy's Campus

Project description:

Anosmia, or loss of the sense of smell, is a core symptom of COVID-19. In most people this sensory dysfunction is fortunately transient, but in many cases COVID can result in persistent anosmia lasting months or years after the initial infection. This project will aim to understand the cellular basis of this persistent deficit, potentially paving the way to new future treatments.

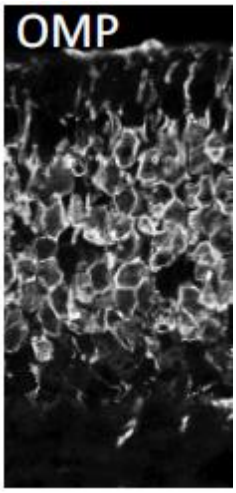


Figure 1. OSNs labelled with OMP in mouse OE (Galliano et al. 2021)

The project will focus on anatomical and cellular characterisation of the olfactory epithelium (OE) in patients with persistent COVID-related anosmia. The OE, located in the nose, is the site of peripheral olfactory sensory transduction. Here, odorant molecules in the breathed air are detected by specialised olfactory sensory neurons (OSNs) and transformed into electrical signals which are sent for processing in the brain. The project will assess the distribution, morphology, activity, proliferation and cell death of OSNs in persistent COVID-related anosmia, to look for key aspects of histopathology which may define the condition.

The project will primarily work with human tissue biopsies taken from patients with persistent COVID-related anosmia during septorhinoplasty surgical treatment. This is in collaboration with Peter Andrews and colleagues at UCLH, who have already secured and prepared all of the necessary paraffin-embedded samples, along with the ethical approval to study them. The project will involve preparing histological sections of these OE samples, then staining them with fluorescent immunohistochemistry to reveal the presence, distribution and intensity of specific markers for OSN identity (e.g. olfactory marker protein; OMP), neuronal activity (pS6, c-fos), proliferation (Ki67) and cell death (activated caspase 3). Label will be assessed using confocal microscopy and quantitative image analysis in FIJI/Matlab. Prior to work on precious human tissue, all techniques and approaches will first be established in sections of mouse OE (Figure 1).

By the end of the project we aim to have a clear understanding of what happens to OSNs in the OE during persistent COVID-related anosmia, and hope to be able to suggest potential future treatment approaches based on this information.

References:

Galliano et al (2021) Brief sensory deprivation triggers cell type-specific structural and functional plasticity in olfactory bulb neurons. J Neurosci, 41:2135-51.

Brann et al. (2020). Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Sci Adv, 6:eabc5081.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -50-

Supervisor(s): Sandra Phillips	**E-mail (lead supervisor):** s.phillips@researchinliver.org.uk

Project title: The Impact of Alcohol on Hepatitis B Replication: Analysis of Viral Markers in human-relevant Models of disease.

Research area (keywords): Hepatitis B and Alcohol-related liver disease

Research skills (keywords): PCR, ELISAs, histology and Immunofluorescence staining (IF), microscopy

Faculty/School/Department: School of Immunology and Microbiology

Location: The Roger Williams Institute of Liver Studies, Denmark Hill

Project description:

Hepatitis B (HBV) infection is a significant global health issue. The World Health Organisation (WHO) estimates that 1/3 of the world's population has been infected with 1.5 million new cases yearly. Currently, 254 million individuals live with chronic hepatitis B (CHB), a leading cause of liver fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Alcohol-related liver disease (ALD) is also a growing concern, with 2.4 billion people consuming alcohol and 75 million diagnosed with alcohol use disorder [2] [3]. In 2019, 3.3 million deaths were attributable to unhealthy alcohol consumption which contributed to 30% of liver cancer cases from 1990 to 2015 [4].

Recent findings indicate that people with CHB who consume high volumes of alcohol or suffer from ALD develop an accelerated progression of chronic liver disease (CLD). Alcohol abuse in individuals with CHB increases the risk of liver cancer by 16-fold [5]. However, the mechanisms driving this rapid disease progression remain largely unknown. Some studies suggest that alcohol increases the HBV replication rate, but this has yet to be confirmed in physiologically relevant human-derived models that faithfully recapitulate HBV and ALD diseases.

At the Roger Williams Institute of Liver Studies, we are developing such models using the Precision Cut Liver Slices (PCLS) and Hepatic Spheroids (HS) platforms [6]. PCLS and HS de-novo infected with HBV, and PCLS from individuals with CHB were treated with alcohol, and samples were collected.

The aim of the master's project is to investigate the effect of alcohol on HBV replication. The collected samples will be processed for histology staining to assess tissue and cell viability, quantitative real-time PCR for HBV gene expression, ELISAs, IF staining for cellular and viral markers and microscope imaging.

Figure 2: Haematoxylin and Eosin stains of Hepatic Spheroids (left) and PCLS (right)

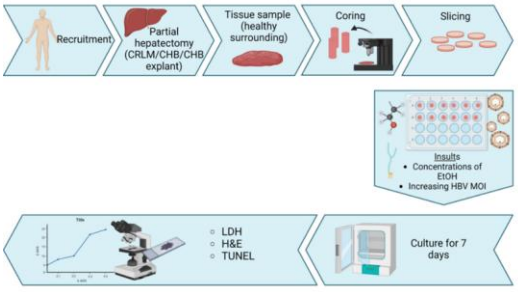


Figure 1: Diagram of the PCLS process

NB: The samples used for this master will be fixed, and RNA will be extracted beforehand to avoid handling infectious material. However, Proof of vaccination against Hepatitis B virus infection is required to work in our facility.

References:[1] Asrani SK, et al. J Hepatol 2019;70:151-171. [2] Liver Disease Crisis. Vol. 2023 (British Liver Trust). [3] Organization, W.H. Global Status Report on Alcohol and Health 2014. Karlsen TH, et al. The Lancet 2022;399:61-116.[4] Akinyemiju T, et al. JAMA Oncol 2017;3:1683-1691. [5] Serfaty L. Gastroenterology 2016;150:1718-1722. [6] Palma E, Doornebal EJ, Chokshi S. Hepatology international 2019;13:51-57.

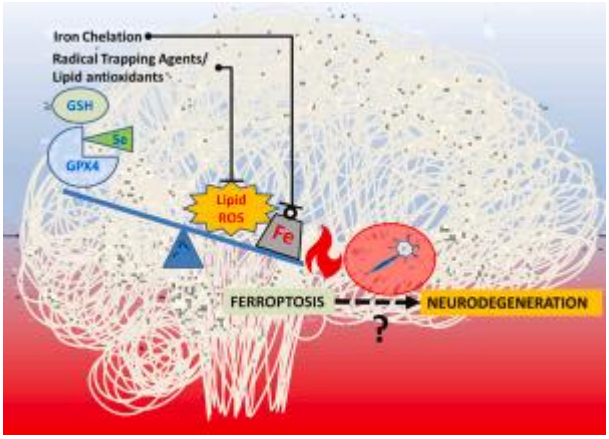
MSc/MRes Biomedical & Molecular Sciences Research

Research Project -51-

Supervisor(s): Dr Yemisi Latunde-Dada	**E-mail (lead supervisor):** yemisi.latunde-dada@kcl.ac.uk
Project title: Studies of compounds that protect against ferroptosis in neuroblastoma cells	
Research area (keywords): Iron Metabolism and Biochemistry	
Research skills (keywords): Biochemical assay techniques, Western blot analysis, ICP-OES, ICP-MS, Microscopy	
Faculty/School/Department: Department of Nutritional Sciences, School of Life Course Sciences	
Location: Franklin Wilkins Building	

Project description:

The accumulation of excessive iron is a characteristic feature of many neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. This leads to oxidative stress and lipid peroxidation, which are observed in these diseases. Antioxidants such as polyphenols, vitamins, and iron chelators can help neutralize the radicals produced by iron toxicity and prevent the formation of phospholipid hydroperoxides. Our project aims to study the effects of these agents on ferroptosis in neuroblastoma cells. Moreover, we will use ferrostatin-1 (Fer-1), an inhibitor of ferroptosis, to understand the functions of these protective compounds in the cell death process. We will assess cell viability using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and utilize Western blot and ICP-MS analyses in the study.



Iron metabolism and ferroptosis in neurodegeneration (2)

References: (1) Kajarabille N, Latunde-Dada GO. (2019) Programmed Cell-Death by Ferroptosis: Antioxidants as Mitigators. Int J Mol Sci. 8;20(19).

(2) Masaldan S, Bush AI, Devos D, Rolland AS, Moreau C. (2019) Striking while the iron is hot: Iron metabolism and ferroptosis in neurodegeneration. Free Radic Biol Med. 133:221-233. https://www.sciencedirect.com/science/article/pii/S0891584918316800

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -52-

Supervisor(s): Dr Yemisi Latunde-Dada	**E-mail (lead supervisor):** yemisi.latunde-dada@kcl.ac.uk
Project title: Content and Availability of minerals in Microgreen Vegetables	
Research area (keywords): Iron Metabolism and Biochemistry	
Research skills (keywords): Biochemical assay techniques, Western blot analysis, ICP-OES, ICP-MS, Microscopy	
Faculty/School/Department: Department of Nutritional Sciences, School of Life Course Sciences	
Location: Franklin Wilkins Building	

Project description:

Mineral deficiencies, particularly iron and zinc, are common in most countries due to inadequate dietary intake. The vulnerable groups are infants, children, and women in their reproductive years. Consequently, debilitating consequences include retarded cognition, reduced physical performance, poor pregnancy outcomes, maternal deaths, and other health problems.

Microgreens are a novel form of cultivating young green seedlings or shoots as vegetables for food. Microgreen vegetables are reputed to contain significantly higher levels of nutrients than their mature counterparts. The project aims to analyse the mineral content of microgreen vegetables at different stages of growth and evaluate their bioavailability using both in vitro and in vivo methods of analysis (1). Further analysis of iron absorption mechanisms will be investigated in Caco-2 cells. The student will gain experience in nutrient analysis techniques and in vitro cellular bioavailability models.

Microgreen vegetables, red amaranth, watercress, beetroot and radish (2)

References: (1) Khoja KK, Buckley A, Aslam MF, Sharp PA, Latunde-Dada GO. (2020) In Vitro Bioaccessibility and Bioavailability of Iron from Mature and Microgreen Fenugreek, Rocket and Broccoli. Nutrients. 10;12(4).

(2) https://www.growjourney.com/how-to-grow-microgreens/

MSc/MRes Biomedical & Molecular Sciences Research

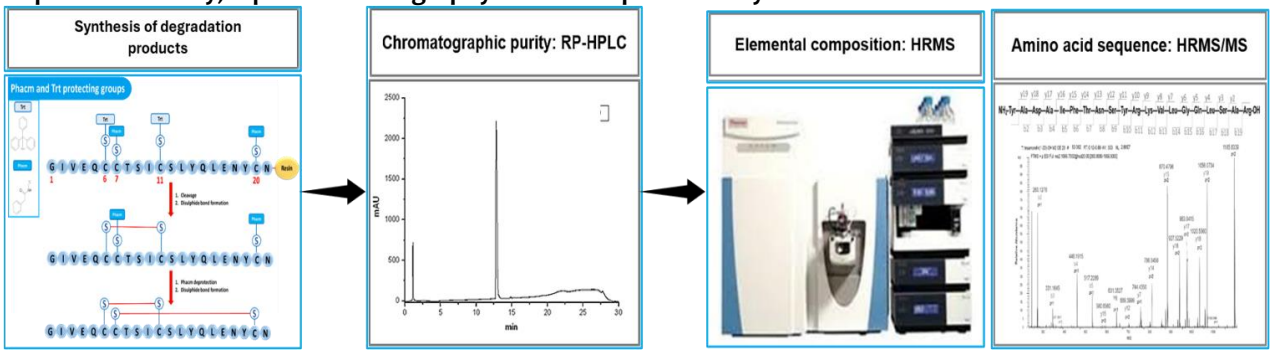
Research Project -53-

Supervisor(s): Dr. Siham Memdouh, Prof. David Cowan and Dr. Vincenzo Abbate	**E-mail (lead supervisor):** Vincenzo.abbate@kcl.ac.uk
Project title: Towards reliable measurement of insulin in blood: Regioselective disulphide bond synthesis of degradation products of insulins	
Research area (keywords): Synthesis. Analytical chemistry. Bioanalysis	
Research skills (keywords): Peptide synthesis, peptide purification by LC-MS, peptide characterisation by HRMS/MS	
Faculty/School/Department: Faculty of Life science and medicine / Institute of Pharmaceutical Sciences/AEFS	
Location: Franklin Wilkins Building, Waterloo Campus	

Insulin is unstable in both sample collection devices and biological matrices disappearing rapidly [1]. Synthetic insulins appear to suffer from the same instability problems. Reliable measurements of these peptide hormones in biological matrices are still challenging for anti-doping and other forensic laboratories. Our previous studies investigated the causes of this disappearance in the preanalytical phase. As a result, degradation products were identified by liquid chromatography high-resolution mass spectrometry (LC-HRMS) in haemolysed and whole blood. A number of these degradation products have been successfully synthesised. This project aims to extend the work to synthesise additional degradation products to use them as diagnostic markers for insulin detection in blood.

As some of the identified degradants contain 4 cysteines, such as the intact A-chain and its degradants, three isomers are possible. This project will focus on the chemical synthesis of pure isomers by applying a regioselective disulphide bonding at specific cysteines. Other degradants contain only two cysteines, and their synthesis is expected to be more straightforward. All degradants will be synthesised by using an automated peptide synthesiser. Preparative LC-MS will be used for peptide purification then LC-HRMS will be used to measure the accurate mass and determine the elemental composition of each synthesised peptide. This will be followed by a full characterisation using MS/MS to prove the correct peptidic sequences.

Synthesised peptides will be used as reference materials to improve the analytical methods used to prove the administration of insulins for anti-doping purposes or the investigation of suspicious deaths associated with insulin overdose. In the future, the approach might also be a useful tool in quantifying the insulins and for clinical applications. This is a multidisciplinary project offering diverse training on chemical synthesis, regioselective disulphide chemistry, liquid chromatography and mass spectrometry.



References:

1. Wunder, C., G.F. Kauert, and S.W. Toennes, Factors leading to the degradation/loss of insulin in postmortem blood samples. Forensic Science International, 2014. 241: p. 173-177.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -54-

Supervisor(s): Dr Adrian Najer **Dr Rui Pedro Galão**	**E-mail (lead supervisor):** adrian.najer@kcl.ac.uk
Project title: Antiviral nanomedicines – creating a coronavirus entry inhibitor	
Research area (keywords): nanomedicine, viral inhibitor, competitive binders, biomimicry	
Research skills (keywords): nanoparticle engineering, dynamic light scattering, UV-Vis spectroscopy, cell culture, lentivirus production, antiviral assays	
Faculty/School/Department: Faculty of Life Sciences & Medicine/School of Cancer & Pharmaceutical Sciences/Institute of Pharmaceutical Science	
Location: FWB Waterloo (nanoparticle work) and Guy's campus (antiviral assays)	

Project description:

Infectious diseases caused by viruses pose an immense burden on global health, as evidenced by coronavirus disease (COVID-19), especially in the absence of broad-spectrum antiviral treatments. Viruses require human host cells for propagation. Hence, interfering with the first step of host cell entry is a promising strategy for developing therapeutic approaches. Nanoparticles (NPs) that interact directly with the extracellular virus and disturb the host cell infection mechanism are emerging candidates for such a broad-spectrum intervention strategy. We have previously identified a potent nano-sized inhibitor of HSV-2, with some activity found against SARS-CoV-2.[1] However, given the limited efficacy against SARS-CoV-2, further optimisation of the NPs through design changes are required before advancing to *in vivo* testing. Very recently, we observed that the backbone structure of the polymers, hence, the particle deformability, plays an important role in modulating efficacy (unpublished).

In this project, various types of NPs with varying degrees of backbone flexibilities will be synthesised using commercial NP building blocks, which are then modified with AMBS (5-amino-2-methoxybenzenesulfonic acid) to present heparan sulphate-mimicking moieties on their surface. The student will analyse the various sets of NPs in terms of size (dynamic light scattering) and surface functionality (zeta-potential, Farndale assay). The student will then learn how to culture the host cells and perform viral inhibition tests using lentiviral particles coated with SARS-CoV-2 spike proteins (in Dr Galão's lab at the Department of Infectious Diseases). These non-replicative lentiviral particles consisting of HIV-Gag Pol proteins encapsulating a firefly luciferase gene and pseudo-typed with representative SARS-CoV-2 spikes (ancestral and variants of concern) will be produced in HEK293T-17 cells. The various nanoparticles will then be tested for their viral inhibition properties using the learned assay.

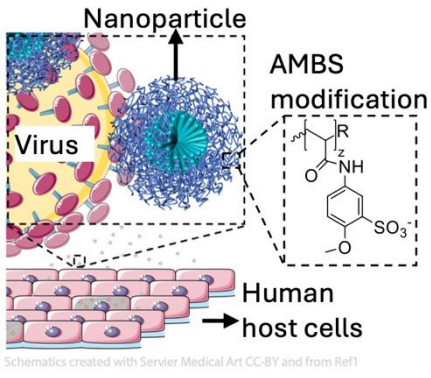


Figure 1. Schematic of antiviral nanomedicine. Polymeric NPs (blue) with various backbone structures will be assembled and modified with AMBS. This creates NPs that mimic host cell (pink) heparan sulphate receptors. Hence, these NPs function as competitive binders for the virion (yellow) spike protein (red). Surface binding of NPs inhibits entry of the virus into their host cell, stopping the infection cycle.

References:

1. Najer, A. *et al.* Potent Virustatic Polymer–Lipid Nanomimics Block Viral Entry and Inhibit Malaria Parasites In Vivo. *ACS Cent Sci* **8**, 1238–1257 (2022).

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -55-

Supervisor(s): **Paul Caton**	**E-mail (lead supervisor):** paul.w.caton@kcl.ac.uk

Project title: Identifying novel targets to treat liver fibrosis and hepatocellular carcinoma

Research area (keywords): liver cancer, liver fibrosis, eNAMPT, fatty liver disease, liver cirrhosis

Research skills (keywords): cell culture, qPCR, immunohistochemistry, transcriptomics

Faculty/School/Department: School of Cardiovascular and Metabolic Medicine and Sciences

Location: Dept of Diabetes and Obesity, Guy's Campus

Project description:

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for around 90% of cases. HCC is thought to effect around £1 million people globally, making it the 5th most common type of cancer worldwide. HCC incidence and related mortality are increasing rapidly (1).

Numerous drugs are available to treat HCC, however typically only around 20-25% of patients achieve disease remission. Novel therapies are required to treat HCC and improve patient outcomes.

Metabolic-dysfunction associated steatohepatitis (MASH) is characterised by fibrosis and inflammation in the liver and is a major risk factor for developing HCC. Our previous work has suggested that eNAMPT (2,3), a protein found in blood, may play a role in driving liver inflammation and fibrosis and may consequently play a role in mediating onset of HCC. Consequently, blocking the action of eNAMPT may be an attractive therapeutic approach to treat HCC (4).

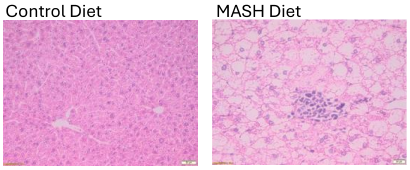


Figure 1: Liver histology image comparing hepatic steatosis in livers of mice fed a control diet, and mice fed a MASH-inducing high-fat, high sugar, high-cholesterol diet.

This project will expand on these findings, with the aim of fully characterising the importance of eNAMPT in driving liver fibrosis and HCC onset and determining whether novel drugs can block the action of eNAMPT can be used to treat HCC.

This project will use a combination of cell culture and disease models of MASH and HCC to:

1. Elucidate the mechanistic effects of eNAMPT on markers of liver fibrosis, inflammation and HCC

2. Determine whether blocking eNAMPT can protect against liver fibrosis, inflammation and HCC

This project will generate novel information characterising pathophysiological mechanisms of disease, as well as determining whether drugs blocking eNAMPT represent a novel therapeutic approach.

Techniques: Cell culture models, tissue sampling, qPCR, immunohistochemistry, transcriptomics

References:

(1) Llovet et al., *Nature Reviews Disease Primers* volume 7, Article number: 6 (2021)
(2) Sayers et al., Diabetologia. 2020 Feb;63(2):313-323.
(3) Kieswich et al., Diabetologia. 2016; 59(11): 2477–2486.
(4) Grolla et al., Br J Pharmacol. 2016 Jul; 173(14): 2182–2194

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -56-

Supervisor(s): Sandra Phillips	**E-mail (lead supervisor):** s.phillips@researchinliver.org.uk

Project title: Unravelling the role of host protein Osteopontin in regulating anti-tumour response to hepatocellular carcinoma during checkpoint receptor inhibition

Research area (keywords): Hepatocellular carcinoma, Hepatitis B

Research skills (keywords): Histology, tissue immunostaining, microscopy, ELISA

Faculty/School/Department: FoLSM/ School of Immunology & Microbial Sciences

Location: The Roger Williams Institute of Hepatology-Foundation for Liver Research, Denmark Hill

Project description: Hepatocellular carcinoma (HCC) represents one of the most common and lethal malignancies globally ranking as the fourth leading cause of cancer-related deaths[1]. Immune checkpoint inhibition (ICI) has revolutionised the treatment landscape for many pathologies including HCC[2]. However, despite significant advances, the response rate to these therapies remains relatively modest (20-30%). Therefore, there is an urgent need to identify the immune alterations that may be responsible for treatment failure to ICI in HCC. Osteopontin (OPN) is a phosphoglycoprotein which overexpression has been linked to poor prognosis in HCC patients[3]. Early reports indicate that OPN drives HCC proliferation, survival and metastases and displays immunomodulatory properties. However, its role in regulating anti-tumour responses to HCC and during checkpoint receptor inhibition is unknown.

Project aims/objectives: To dissect the role of OPN within HCC (on viral and non-viral backgrounds) tumour immune microenvironment and investigate its association with immune checkpoints.

1) Precision cut tumour slices (PCTS) obtained from HCC resections were subjected to various treatments: untreated, recombinant OPN, OPN APTAMER and SHAM control APTAMER. These treatments were also combined with and without anti-PD1 monoclonal antibody Nivolumab. The samples will undergo the following analysis:

    a. PCTS histology, apoptosis, proliferation and immune markers will be assessed. Cell death will also be quantified in the PCTS culture supernatants.

    b. Supernatants collected from untreated and Nivolumab treated PCTS cultures will be used to quantify OPN, chemokines and cytokines.

2) We have collected longitudinal samples from HCC patients treated with Nivolumab in the BMS 904060 clinical trial. Serum will be quantified for OPN in stable disease.

Figure 1: PCTS

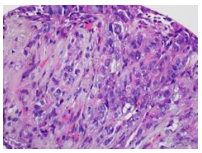


Figure 2: PCTS histology staining

NB: Proof of vaccination against Hepatitis B virus infection is required to work in our building.

References: 1. Yang C, Zhang H, et al. Evolving therapeutic landscape of advanced hepatocellular carcinoma. *Nature Reviews Gastroenterology & Hepatology* 2023;20(4):203-22. **2.** Llovet JM, Castet F, et al. Immunotherapies for hepatocellular carcinoma. *Nature Reviews Clinical Oncology* 2022;19(3):151-72. **3.** Kariya Y, Kariya Y. Osteopontin in Cancer: Mechanisms and Therapeutic Targets. *International Journal of Translational Medicine* 2022;2(3):419-47.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -57-

Supervisor(s): Karen Steel; Neil Ingham	**E-mail (lead supervisor):** karen.steel@kcl.ac.uk

Project title: Can progressive hearing loss be reversed?

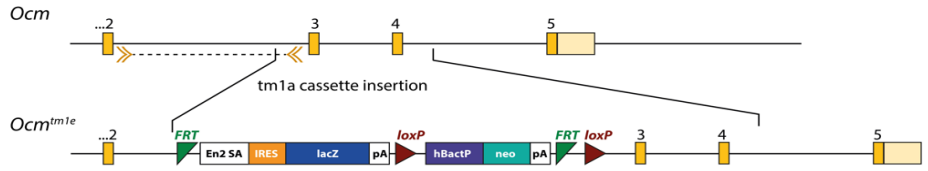
Research area (keywords): Deafness; Mouse; Reversing pathology; Physiology; Calcium buffer

Research skills (keywords): Working with mice; Mouse genetics; PCR for genotyping; Hearing assessment; Fine dissection of the cochlea; Immunolabelling and confocal microscopy; Data analysis; Report writing; Presentation skills.

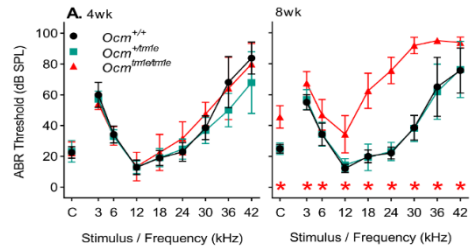
Faculty/School/Department: IoPPN/Neuroscience/Wolfson Sensory, Pain and Regeneration Centre.

Location: Guy's campus

Project description: The project involves investigating whether hearing loss due to the *Ocm*^{tm1e} mutation can be reversed by activating the gene using the same approach used for the *Spns2* mutation described in Martelletti et al 2023. Oncomodulin, encoded by *Ocm*, is a calcium buffer strongly expressed in outer hair cells (OHCs) of the cochlea. Knockout of the gene leads to progressive increase in auditory thresholds, starting between 4 and 8 weeks of age. The structure of the mutation allows us to correct the mutation and activate transcription of the gene at various ages after the onset of the hearing loss, allowing us to ask if this type of hearing loss can be reversed. In this project, auditory function will be assessed using Auditory Brainstem Responses (ABR) in anaesthetised mice before and at intervals following activation of the *Ocm* gene (training in using animals in research will be provided and the student will obtain a Personal Licence for this part of the project). The efficiency of the restoration of gene transcription (PCR-based method) and translation (immunolabelling) will be assessed and molecular techniques also used for identifying the required genotypes of mice to use.



Above: Diagram of the *Ocm*^{tm1e} mutation: Top, normal *Ocm* gene, exons in yellow. Bottom, *Ocm*^{tm1e} mutation showing large DNA insertion that disrupts transcription leading to hearing loss. The gene is reactivated by exposure to Flp recombinase, an enzyme that recombines the sequence between the FRT sites (green triangles) and removes the inserted DNA, restoring gene transcription.



Left: ABR thresholds from *Ocm*^{tm1e} homozygotes (red), heterozygotes (green) and normal wildtype mice (black) at 4 weeks and 8 weeks old, showing mutants have normal thresholds at 4 weeks but by 8 weeks their thresholds have increased indicating progressive hearing loss.

References: Martelletti et al. Reversal of an existing hearing loss by gene activation in Spns2 mutant mice. PNAS 120:e2307355120

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -58-

Supervisor(s): Karen Steel; Dan Pentland	**E-mail (lead supervisor):** karen.steel@kcl.ac.uk

Project title: Can correcting expression of a mutant gene stop sensory hair cell degeneration?

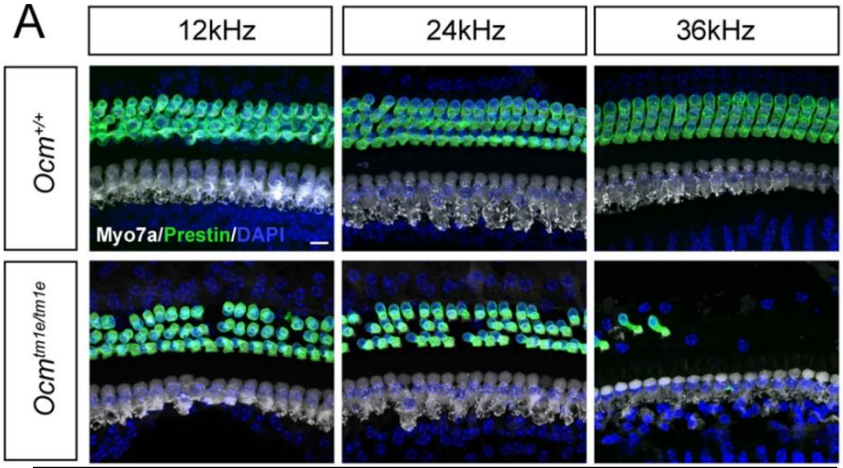
Research area (keywords): Deafness; Mouse; Reversing pathology; Physiology; Calcium buffer

Research skills (keywords): Working with mice; Mouse genetics; PCR for genotyping; Assessment of expression levels in tissues; Fine dissection of the cochlea; Immunolabelling and confocal microscopy; Data analysis; Report writing; Presentation skills.

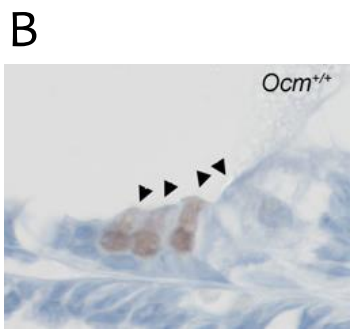
Faculty/School/Department: IoPPN/Neuroscience/Wolfson Sensory, Pain and Regeneration Centre.

Location: Guy's campus

Project description: The project involves investigating whether hearing loss due to the *Ocm*^{tm1e} mutation can be reversed by activating the gene using the same approach used for the *Spns2* mutation described in Martelletti et al 2023. Oncomodulin, encoded by *Ocm*, is a calcium buffer strongly expressed in outer hair cells (OHCs) of the cochlea. Knockout of the gene leads to progressive increase in auditory thresholds, starting between 4 and 8 weeks of age. The structure of the mutation allows us to correct the mutation and activate transcription of the gene at various ages after the onset of the hearing loss, allowing us to ask if this type of hearing loss can be reversed. This project would involve assessing the effects of activation of the *Ocm* gene on the organ of Corti, by assessing changes in hair cell survival using immunolabelling and confocal analysis. The efficiency of the restoration of gene transcription (PCR-based method) and translation (immunolabelling) will be assessed and molecular techniques also used for identifying the required genotypes of mice to use.



A.Top, Control, 3 rows of OHCs (green) and 1 row of inner hair cells (white). Bottom, Mutant, gaps indicate loss of OHCs.



B. Section of an immature organ of Corti showing brown immunolabelling of OHCs. Arrowheads point to inner hair cells and OHCs.

References: Martelletti et al. Reversal of an existing hearing loss by gene activation in Spns2 mutant mice. PNAS 120:e2307355120

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -59-

Supervisor(s): Dr. Shobha Ahi	**E-mail (lead supervisor):** shobha.ahi@kcl.ac.uk
Project title: Excretion profiles of methylphenidate and its metabolites in humans	
Research area (keywords): Analytical Toxicology, Clinical Toxicology, drug of abuse testing, marker metabolite identification	
Research skills (keywords): wet chemistry techniques, LLE, SPE, LC-MS/MS, biological specimen	
Faculty/School/: School of Cancer & Pharmaceutical Sciences, Faculty of Life Sciences & Medicine	
Location: Drug Control Centre, FWB, King's College London	

Project description:

Background: Methylphenidate (MP), is commonly prescribed to treat attention deficit/hyperactivity disorder (ADHD) in the UK. It is banned in sports as a stimulant and is the most frequently reported of this class. MP is modified chemically (metabolised) following administration to ritalinic acid (RA). Concurrent administration of MP with alcohol affects the urinary concentrations of MP and RA and may therefore affect their reporting of results in an athlete's sample. This project aims to study the urinary excretion profile of Methylphenidate and its metabolites relevant to doping control analysis in urine samples of athletes.

Deliverables:

1. Identification of additional specific target metabolite/s of MP in human urine. An optimized method of analysis using LC-MS/MS which can be applied to routine doping control samples for interpretation of results.
2. Establishing the effect of alcohol on the urinary concentration of MP, RA and EP which is critical for interpretation of analytical data in context to anti-doping.

Techniques and Laboratory Skills:

The postholder will learn skills that will equip them for a future in toxicology and drug testing laboratories. These include: handling of human biological samples, sample extraction using liquid-liquid extraction and solid phase extraction techniques, handling of LC-MS/MS instrument and analysis of analytical data including quality controls.

Development Opportunities and Skills

This is an exciting opportunity to be involved in anti-doping science. The post-holder will contribute to the Drug Control Centre's and King's Forensics research activities, prepare and present submissions to national and international conferences and develop this work into a significant publication. The role will be based in the Drug Control Centre at King's which is the only accredited laboratory for sports drug testing in the UK.

References:

1. PharmGKB summary: Methylphenidate Pathway, Pharmacokinetics/Pharmacodynamics, T.Stevens, K. Sangkuhlc , J. T. Brown, R. B. Altmanc, T.E. Klein, Pharmacogenet Genomics. 2019; 29(6): 136–154

2. Identification of in vitro metabolites of ethylphenidate by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, N.Negreira, C. Erratico, A. L.N. van Nuijs, A. Covac, Journal of Pharmaceutical and Biomedical Analysis 117 (2016) 474–484

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -60-

Supervisor(s): Prof. Kim Wolff and Dr. Shobha Ahi	**E-mail (lead supervisor):** shobha.ahi@kcl.ac.uk
Project title: The analysis of steroid esters in dried blood spots (DBS) samples for administration of pseudoendogenous steroids	
Research area (keywords): Analytical Toxicology, Clinical Toxicology, DBS, drug of abuse testing	
Research skills (keywords): DBS handling, LLE, SPE, LC-MS/MS, biological specimen	
Faculty/School/: School of Cancer & Pharmaceutical Sciences, Faculty of Life Sciences & Medicine	
Location: Drug Control Centre, FWB, King's College London	

Project description:

Background: Drug testing is essential for various purposes, including forensic investigations, clinical monitoring, and workplace safety. Traditional methods, such as urine and blood tests, have limitations, such as invasiveness and sample collection challenges. Dried blood spots (DBSs) offer an attractive alternative due to their ease of collection, stability, and cost-effectiveness. The endogenous steroid testosterone has been prohibited in sport by the World Anti-Doping Agency (WADA) for over 40 years but has remained one of the most widely abused substances by athletes seeking to gain a competitive advantage due to its anabolic properties and wide availability. The detection of steroid esters in DBS would unequivocally prove the administration of psuedoendogenous steroids such as testosterone, nandrolone and boldenone without the need for steroid profiling or GC-C-IRMS analysis.

Deliverables:

1. A LC-MS/MS method for the detection of steroid esters in dried blood spots (DBS) samples.
2. To determine whether DBS can be routinely used for the detection of steroid esters in DBS for application of sports testing.

Techniques and Laboratory Skills:

The postholder will learn skills that will equip them for a future in toxicology and drug testing laboratories. These include: handling of human biological samples including DBS, sample extraction using liquid-liquid extraction and solid phase extraction techniques, handling of LC-MS/MS instrument and analysis of analytical data including quality controls.

Development Opportunities and Skills

This is an exciting opportunity to be involved in anti-doping science. The post-holder will contribute to the Drug Control Centre's and King's Forensics research activities, prepare and present submissions to national and international conferences and develop this work into a significant publication. The role will be based in the Drug Control Centre at King's which is the only accredited laboratory for sports drug testing in the UK.

References:

1. AD Brailsford, WNM Majidin, N Wojek, DA Cowan, C Walker IRMS delta values (13C) of nandrolone and testosterone products available in the UK: Implications for anti-doping. Drug testing and analysis 2018 Nov;10(11-12):1722-1727. doi: 10.1002/dta.2530.

2. Thevis M, History of sports drug testing, in: Mass Spectrometry in Sports Drug Testing – Characterization of Prohibited Substances and Doping Control Analytical Assays John Wiley & Sons, 2010, p. 19.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -61-

Supervisor(s): Dr Simon Cleary	**E-mail (lead supervisor):** simon.cleary@kcl.ac.uk

Project title: Modulating pulmonary vascular function to improve lung health

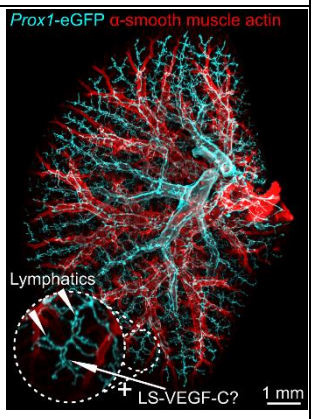
Research area (keywords): Immunology, lung diseases, pharmacology

Research skills (keywords): Imaging, in vivo, immunofluorescence

Faculty/School/Department: Life Sciences & Medicine, Institute of Pharmaceutical Science

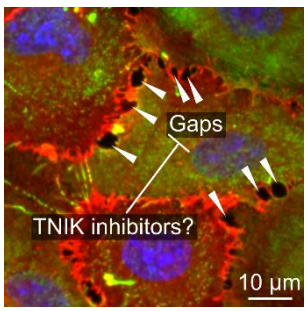
Location: Guy's campus

Project description: **Background:** Blood and lymphatic vessels regulate immune responses and fluid balance in the lungs. My research aims to find ways to modulate the functions of these vessels to improve lung disease outcomes.



Project 1: Therapeutic modulation of pulmonary lymphatics. Context: Promoting lung lymphatic function to increase removal of inflammatory mediators and immune cells from the lungs might help accelerate resolution of several lung diseases (**1**). VEGF-C drives lymphatic growth by activating VEGFR3, but this effect of VEGF-C has not yet been exploited therapeutically in lung diseases. In part, this is because VEGF-C also activates VEGFR2 leading to undesirable formation of leaky blood vessels. Collaborator Eric Song (Yale) has developed lymphatic specific (LS-)VEGF-C, a new potent activator of VEGFR3 that does not bind VEGFR2. This project will aim to: **(i) test whether inhaled delivery of LS-VEGF-C can promote lung-restricted lymphangiogenesis using new imaging approaches (right) and (ii) enhance pulmonary lymphatic function without causing vascular leakage.** These studies will enable future investigations into whether LS-VEGF-C treatment has therapeutic efficacy in lung disease models.

Project 2: Re-sealing leaky blood vessels in the lungs. Context: Endothelial gaps open during inflammation (right), leading to vascular leak. In injured lungs this response can cause fatal pulmonary oedema. Collaborator Aleksandar Ivetic (King's) has found that an inhibitor of TNIK can reseal gaps formed between human umbilical vascular endothelial cells in vitro. Another group identified that another TNIK inhibitor can reduce pulmonary fibrosis in mice (**2**), but it is unclear whether TNIK inhibition can reduce lung vascular permeability. This project will aim to **(i) Determine whether pharmacologic inhibitors of TNIK can reduce lung vascular permeability in models of acute lung injury (antibody-mediated and secondary to influenza A virus infection (3,4), and (ii) test whether these treatments improve lung function (SpO_2) in these models.** These studies will determine whether TNIK inhibition could be a useful strategy for treating acute lung injury.



Approaches: Immunofluorescence, fluorescence microscopy, image analysis (ImageJ, Imaris), dye tracing of vascular function, pulse oximetry.

Environment: Based in the new King's Centre for Lung Health, an interdisciplinary centre focused on lung health & disease. Specialist microscopy training will be provided through the King's Nikon Imaging Centre.

Required skills: Some liquid handling experience, enthusiasm to learn and to apply for scholarships.

References: (1) doi.org/10.3389/fmed.2023.1118583; (2) doi.org/10.1038/s41587-024-02143-0; (3) doi.org/10.1172/JCI178351; (4) doi.org/10.1152/ajplung.00046.2023

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -62-

Supervisor(s): Dr Antonio Riva Miss Phoebe Tsou	**E-mail (lead supervisor):** a.riva@researchinliver.org.uk

Project title: *Sex control of peripheral immunity during alcohol-related liver disease*

Research area (keywords): liver disease, alcohol, sex-based differences, immunology

Research skills (keywords): handling of human blood samples, plasma/serum separation, uniplex/multiplex quantification assays, biostatistics

Faculty/School/Department: The Roger Williams Institute of Hepatology, affiliated with the Faculty of Life Sciences and Medicine / School of Immunology and Microbial Sciences / Department of Inflammation Biology

Location: The Roger Williams Institute of Hepatology, Denmark Hill, 111 Coldharbour Lane, London SE5 9NT

Project description:

Background. Females have naturally stronger immune responses than males. This helps to protect females from infections and diseases, but it can also be a hindrance during immune-mediated conditions like autoimmune diseases, inflammation and cytokine storms, which are typically more prevalent or intense in females than in males.

Alcohol-related liver disease (ArLD) is associated with well-documented immunological changes, including persistent systemic hyper-inflammation and progressive loss of antibacterial defences. Immunopathogenesis is a key driver of disease progression and outcome in ArLD patients, including increased susceptibility to bacterial infection, liver decompensation, organ failure and death.

Although the consequences of alcohol consumption are generally more severe in females, and there may also be baseline disparities in societal exposure to alcohol across sexes, how sex-related immune differences contribute to distinct disease trajectories in males and females remains unclear.

Aim. This project will investigate how sex influences immunity and inflammation in a variety of patients at different stages of ArLD, ranging from drinking individuals without liver disease to patients with severe alcoholic hepatitis, the most inflammatory and life-threatening form of ArLD.

Technical aspects. The student will learn cutting-edge technologies such as high-sensitivity electrochemiluminescence multiplex ELISAs, Luminex, and potentially flow cytometry in a fully equipped state-of-the-art laboratory at the Roger Williams Institute of Hepatology, a purpose-built internationally renowned research centre entirely dedicated to translational research in liver disease. Biological samples, instrumentation and a solid technical and statistical support are available at the Institute. Besides wet-lab experimental procedures, the candidate will also be involved in data analysis and possibly setting up novel protocols, which will be a stimulating challenge and will help the development of detailed problem-solving skills and critical thinking.

References: Riva A. Front Immunol 14:1178541 (2023). Lotersztajn S, Riva A, et al. Z Gastroenterol 60, 58-66 (2022). Riva A, et al. Front Physiol 12:632502 (2021). Sharma L, Riva A. Microorganisms 8(11):1744 (2020). Riva A and Gray EH, et al. JHEP Rep 2, 100151 (2020). Riva A, et al. Gut 67, 918-930 (2018). Riva A, Chokshi S. Hepatol Internat 12(3):223-236 (2018). Markwick LJ, Riva A, et al. Gastroenterology 148, 590-602.e510 (2015).

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -63-

Supervisor(s): Dr Antonio Riva Miss Phoebe Tsou	**E-mail (lead supervisor):** a.riva@researchinliver.org.uk

Project title: *Immunological markers of no response to single-agent immunotherapy during severe alcoholic hepatitis*

Research area (keywords): liver disease, alcohol, immunology, immunotherapy

Research skills (keywords): handling of human blood samples, plasma/serum/cell separation, cell culture, uniplex/multiplex quantification assays, flow cytometry, biostatistics

Faculty/School/Department: The Roger Williams Institute of Hepatology, affiliated with the Faculty of Life Sciences and Medicine / School of Immunology and Microbial Sciences / Department of Inflammation Biology

Location: The Roger Williams Institute of Hepatology, Denmark Hill, 111 Coldharbour Lane, London SE5 9NT

Project description:

Background. Alcohol-related liver disease (ArLD) is linked to well characterised immune alterations, including chronic systemic hyper-inflammation and progressive loss of antibacterial responses. Immunopathogenesis is a key driver of disease progression and outcome particularly in patients with Severe Alcoholic Hepatitis (SAH), the most inflammatory and life-threatening form of ArLD, including increased susceptibility to bacterial infection, liver decompensation, organ failure and death.

Single-agent immunotherapy against inflammatory pathways, such as the IL-1 pathway, has been investigated in several randomised clinical trials as a novel strategy to dampen unwanted injurious inflammation during SAH. However, the results of these trials have been disappointing, suggesting that combination treatments co-targeting multiple immunoregulatory pathways at once could be more promising options in these patients.

Aim. We have collected immune cell samples from patients undergoing one of these trials, and this preliminary project aims to assess immune markers of no response to single-agent immunotherapy treatment and identify co-targetable immunoregulatory pathways to facilitate antibacterial immune recovery in these patients.

Technical aspects. The student will learn and apply technologies such as immune cell cultures, flow cytometry and high-sensitivity multiplex quantification assays (ELISAs, Luminex) in a fully equipped state-of-the-art laboratory at the Roger Williams Institute of Hepatology, a purpose-built internationally renowned research centre entirely dedicated to translational research in liver disease. Biological samples, instrumentation and a solid technical and statistical support are available at the Institute. Besides wet-lab experimental procedures, the candidate will also be involved in data analysis and possibly setting up novel protocols, which will be a stimulating challenge and will help the development of detailed problem-solving skills and critical thinking.

References: Vergis N, et al. Clin Gastr Hep, in press (2024). Riva A. Front Immunol 14:1178541 (2023). Lotersztajn S, Riva A, et al. Z Gastroenterol 60, 58-66 (2022). Riva A, et al. Front Physiol 12:632502 (2021). Sharma L, Riva A. Microorganisms 8(11):1744 (2020). Riva A and Gray EH, et al. JHEP Rep 2, 100151 (2020). Riva A, et al. Gut 67, 918-930 (2018). Markwick LJ, Riva A, et al. Gastroenterology 148, 590-602.e510 (2015).

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -64-

Supervisor(s): Anna Zampetaki	**E-mail (lead supervisor):** anna.zampetaki@kcl.ac.uk

Project title: Blood Vessel Organoids as a model of Diabetic Vascular Dysfunction

Research area (keywords): Gene Editing, Molecular and Cellular Biology, Tissue Engineering

Research skills (keywords): Gene editing, induced pluripotent stem cells, human blood vessel organoids

Faculty/School/Department: : Faculty of Life Sciences and Medicine/ School of Cardiovascular Medicine and Sciences

Location: Denmark Hill Campus

Project description:

Background: Diabetes mellitus has major macrovascular and microvascular complications[1]. Human blood vessel organoids derived from induced pluripotent stem (iPS) cells have recently been developed[2]. They display a striking structural and functional similarity to human blood vessels (Figure 1). Patient-derived organoids can serve as an experimental system to study not only the pathogenesis of the disease, but also to initiate drug screening for effective therapeutic approaches. Genome engineering can be used to rescue the functionality of patient organoids[3,4] as the gene editing platform CRISPR/Cas9 is a powerful technology for repressing gene expression in bulk populations[4,5].

Project: We would like to integrate the iPS technology, genetic engineering and tissue modelling to establish a versatile platform to dissect the mechanisms of diabetic vasculopathy. Our aim is to generate mutant human iPS cells from patients using CRISPR/ Cas9 gene editing system. Should a scholarship be granted for the project, the student will subsequently use these mutant lines to evaluate their angiogenic potential in a human blood vessel organoid system.

Training: The student will gain extensive experience in cutting edge technologies namely, iPS cells, CRISPR/Cas9 gene editing, bioinformatic analysis and molecular biology techniques (DNA isolation, molecular cloning, mutation detection, RNA isolation, reverse transcription and QPCR).

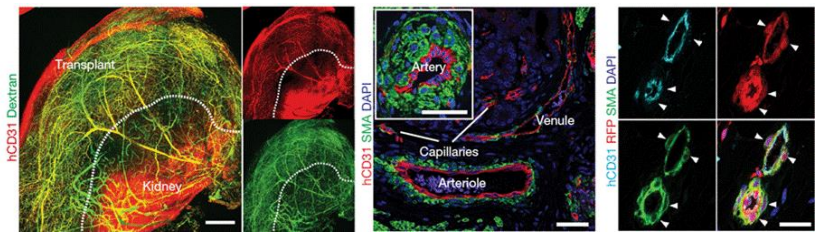


Figure 1 (Wimmer et al Nature

References: **1.** Cade WT. Phys Ther 2008;88:1322–1335 **2.** Wimmer RA et al. Nature 2019; 565: 505-510 **3.** Tuveson D and Clevers H Science 2019; 364: 952-955 **4.** Romeo SG Nat Commun. 2023 Sep 9;14(1):5552 **5.** Lataniotis L et al. Sci Rep. 2017 Aug 17;7(1):8585.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -65-

Supervisor(s): Dr Luca Urbani & **Prof Francesca Spagnoli**	**E-mail (lead supervisor):** luca.urbani@researchinliver.org.uk
Project title: Induced pluripotent stem cell differentiation into pancreatic cells using 3D culture on decellularized fetal scaffolds vs. conventional hydrogels	
Research area (keywords): stem cell, regenerative medicine, pancreas, diabetes, development	
Research skills (keywords): 3D cell culture, iPS cells, decellularisation, immunohistochemistry/ immunofluorescence, protein and gene expression analysis	
Faculty/School/Department: Faculty of Life Sciences & Medicine / School of Immunology & Microbial Sciences /The Roger Williams Institute of Hepatology &	
Location: Denmark Hill campus and Guy's Campus	

Background. Induced pluripotent stem (iPS) cells hold immense potential for regenerative medicine, particularly in the treatment of diabetes through the generation of insulin-producing pancreatic cells. Traditional 2D culture methods limit the efficacy of iPS cell differentiation, necessitating advanced 3D culture systems. Decellularized scaffolds obtained from organs and tissues provide a biomimetic environment that closely resembles native extracellular matrices, offering a promising alternative to conventional hydrogels for cell culture and differentiation.

Aim and objectives. This study aims to compare the efficiency of iPS cell differentiation into pancreatic cells using 3D cultures on decellularized fetal pancreas scaffolds versus normal hydrogels. The project will assess cell viability, differentiation efficiency, and functionality of the derived pancreatic cells.

Methodology

1. Scaffold Preparation: Decellularization of fetal pancreas tissues will be performed, followed by characterization of the scaffold structure and composition. Decellularised scaffolds will be used unprocessed or pulverised to be combined with hydrogels.
2. iPS cells will be cultured on different scaffolds and combination of hydrogels, and subjected to a pancreatic differentiation protocol.
3. Analysis: Differentiation efficiency will be evaluated by analysing markers specific to pancreatic lineage using RTqPCR, FACS and IF as well as functional assays

This study will provide insights into the potential of decellularized fetal pancreas scaffolds to enhance iPS cell differentiation into pancreatic cells, potentially offering a superior platform for pancreatic tissue engineering.

References:

Cozzitorto C, et al. 2020. doi: 10.1016/j.devcel.2020.08.003.

Mueller LM, et al. 2024. doi: 10.1038/s41467-024-46740-8.

MSc/MRes Biomedical & Molecular Sciences Research

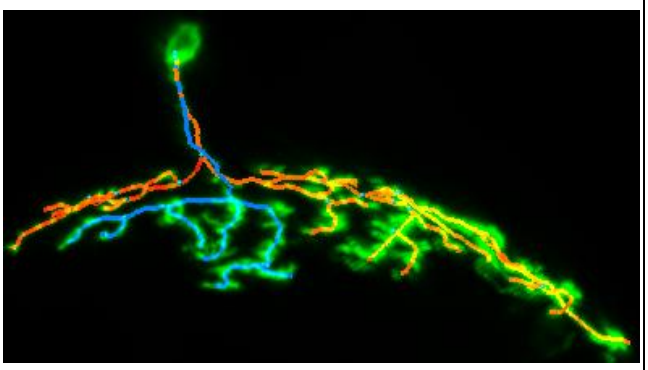
Research Project -66-

Supervisor(s): Prof Robert Hindges	**E-mail (lead supervisor):** robert.hindges@kcl.ac.uk
Project title: Creation of transgenic zebrafish to label specific neuronal subtypes	
Research area (keywords): Neuroscience, Molecular Biology, Development, Vision	
Research skills (keywords): RNA fluorescent in situ hybridization (FISH), PCR, CRISPR genome editing, zebrafish husbandry, embryo injections, confocal microscopy	
Faculty/School/Department: Centre for Developmental Neurobiology, MRC Centre for Neurodevelopmental Disorders, IoPPN	
Location: New Hunt's House, Guy's Campus	

Project description:

Our brain consists of **thousands of different types of neurons** with specific morphologies and functions. To **investigate the exact roles of an individual neuron subtype**, including assessing its distribution and arrangement in the brain, it is **necessary to be able to mark it,** for example with a fluorescent reporter.

My lab is interested in the **different neurons that underly visual function**, in particular amacrine cells in the retina. We have previously identified several different types, but so far, we do not have specific reporters for each of them, but rather use random labelling methods for visualisation. Using single-cell RNAseq methods, **we have now identified several genes to be uniquely expressed** in some of these amacrine subtypes.



This project will use this data as a basis and **1) confirm the expression of the identified genes in different neuronal subtypes** using multiplex RNA-FISH and 2) **construct DNA donors** to insert a reporter into the gene loci and 3) **apply CRISPR-Cas9 gene editing methods** to create transgenic zebrafish after injections at the one cell stage.

The outcome of the project will be the **generation of different transgenic zebrafish driver lines** and- depending on time- a first look at the labelled cells.

For more details on the Hindges Lab, visit www.hindgeslab.org

References:

- Antinucci P, Suleyman O, Monfries C, Hindges R. (2016) Neural Mechanisms Generating Orientation Selectivity in the Retina. Current Biology PMID: 27374343
- Zhang Y, Marshall-Phelps K, de Almeida RG. (2023) Fast, precise and cloning-free knock-in of reporter sequences in vivo with high efficiency. Development PMID: 37309812

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -67-

Supervisor(s): Robert Köchl	**E-mail:** Robert.Koechl@kcl.ac.uk
Project title: Mechanobiological control of T cell dependent immune responses.	
Research area (keywords): Immunology, Cell Biology, mechanobiology	
Research skills (keywords): Cell culture, Flow Cytometry, Super resolution microscopy	
Faculty/School/Department: School of Immunology and Microbial Sciences/Peter Gorer Department of Immunobiology.	
Location: Borough Wing, Guy's Campus	

Project description:

Cytotoxic CD8 T Lymphocytes (CTL) are a critical component of the adaptive immune system and are responsible for clearing virus infected cells and cancer cells by secreting a cocktail of cytolytic enzymes through a specialized structure called the cytolytic synapse. To achieve their anti-cancer function, they need to migrate into tumours, adhere to target cells and kill them. Tumours are, however, often much stiffer than healthy tissue. This increase stems from two factors, firstly the deposition of extracellular matrix and rapid cell proliferation in the tumour, and secondly, increased osmotic stress, which together causes the intra-tumoral pressure to go up. This enhanced stiffness not only inhibits CTLs from entering a tumour, but may also negatively affect their ability to polarize and kill target cells. We believe the main reason for this is that the compression forces inside of the tumours are so high, that CTLs can not change their volume and shape anymore to form a cytolytic synapse (e.g. they get squeezed too much).

This project aims to characterize the effect of physical compression – both osmotic and mechanical – on the ability of CTLs to recognize antigen and form productive cytolytic synapses. Particularly we will analyse how compression affects T cell receptor signalling and actin dynamics, as well as the polarization of the killing apparatus and efficient target cell killing. These aims will be addressed by combination of biochemistry and super-resolution imaging approaches. The student will purify and culture primary mouse CTLs and then use various microscopes, such as TIRF or confocal microscopes to study the location of key components of the killing machinery, such as actin, the microtubule organizing centre and cytotoxic granules within CTL that are either binding to cancer cells or to functionalized glass surfaces. In parallel, the student will use techniques such as immunoblotting and flow cytometry to assess which pathways downstream of the T cell receptor are defective in compressed CTLs.

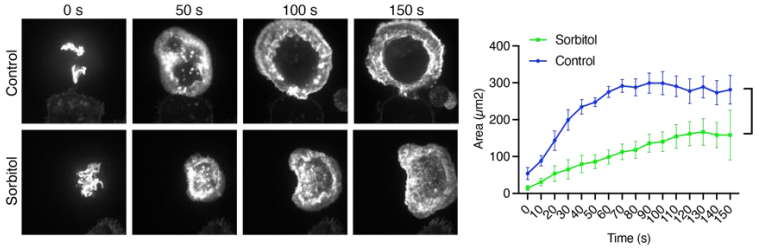


Figure: Osmotic compression of CTLs expressing the filamentous actin marker Lifeact-GFP with sorbitol reduces their ability to spread on activating surfaces.

References:

Köchl R et al. WNK1 kinase balances T cell adhesion versus migration in vivo. Nature Immunology. 2016 Sep;17(9):1075-83.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -68-

Supervisor(s): Prof Katie Doores	**E-mail (lead supervisor):** katie.doores@kcl.ac.uk

Project title: Mechanisms of antibody-mediated hantavirus neutralization

Research area (keywords): Virology, immunology, Emerging virus, neutralizing antibody

Research skills (keywords): FACS, ELISA, tissue culture, molecular biology, protein biochemistry

Faculty/School/Department: Department of Infectious Diseases/SIMS/FoLSM

Location: 2nd Floor, Borough Wing, Guy's Hospital

Project description:

Highly pathogenic animal and arthropod viruses capable of jumping the species barrier pose a significant and continued threat to human health. The recent SARS-CoV-2 global pandemic highlights the pressing need to develop therapeutic strategies to protect against the spread or intentional dissemination of such deadly pathogens. Monoclonal antibodies (mAbs), which target viral glycoproteins displayed on the virion envelope, have become some of the most effective reagents for prevention or treatment of infected individuals, e.g. Palivizumab (Synagis™) for prevention of respiratory syncytial virus (RSV) infection and ZMapp™ (a mAb cocktail) for treatment of Ebola virus infection. Here, we will study antibody responses against hantaviruses, a geographically diverse group of zoonotic pathogens, that cause hantavirus pulmonary syndrome (HPS) or hemorrhagic fever with renal syndrome (HFRS) with morality rates up to 40%.

The aim of this project is to develop mAbs that neutralize genetically diverse hantaviruses for use in treatment and prevention during disease outbreaks. We have previously generated a hantavirus neutralizing mAbs through immunization of rabbits with recombinant hantavirus surface glycoproteins [1] and from survivors of hantavirus infection (Figure 1). Hantavirus specific mAbs were isolated using antigen-specific single B cell sorting of PBMCs. mAbs will be expressed in mammalian cells and purified for characterization. Expressed mAbs will be characterised functionally using ELISA and a cell-based CL2 hantavirus neutralization system and fusion assay. The antibody epitopes will be determined through collaborative structural work and the antibody paratopes mapped using site-directed mutagenesis. These studies will identify mAbs for future animal protection studies and reveal sites of antibody vulnerability that could be exploited for vaccine design.

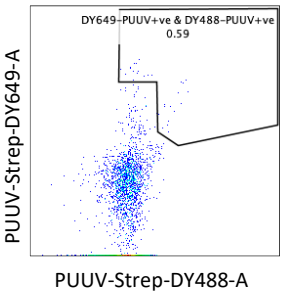


Figure 1: FACS plot showing human B cells that bind hantavirus glycoproteins. mAbs from these B cells will be cloned, expressed and characterised.

References:

1. I. Rissanen et al, Structural basis for a neutralizing antibody response elicited by recombinant Hantaan virus Gn immunogen, mBio, 2021, 12(4):e0253120. doi: 10.1128/mBio.02531-20.

2. E.R. Allen et al, A protective monoclonal antibody targets an immunodominant site of vulnerability on the surface of Rift Valley fever virus, Cell Reports, 2018, 25(13):3750-3758.e4.

MSc/MRes Biomedical & Molecular Sciences Research

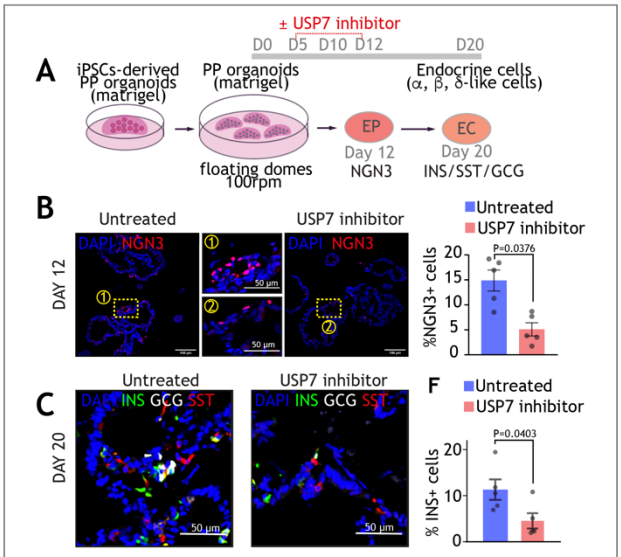
Research Project -69-

Supervisor(s): Rocio Sancho	**E-mail (lead supervisor):** rocio.sancho@kcl.ac.uk
Project title: Characterization of USP7-NGN3 overexpressing iPSCs cells	
Research area (keywords): diabetes, pluripotent stem cells, beta cell regeneration	
Research skills (keywords): iPSCs culturing and differentiation, molecular biology, Crispr-Cas9, confocal microscopy, flow cytometry (FACS)	
Faculty/School/Department: Life Sciences and Medicine, Basic and Medical Bioscience School, Centre for Gene Therapy & Regenerative Medicine	
Location: 28th Floor – Tower Wing – Guy's Hospital	

Project description:

In the last decade, stem cell research has shed new light on novel regenerative therapies for diabetes. Efficient protocols have been described to induce differentiation of induced pluripotent stem cell (iPSCs) into insulin-producing beta-like cells. However, the process is still inefficient, and the beta-cells generated are often not fully functional. One of the key factors required for the success of the beta cell differentiation process is Ngn3. We have recently identified the deubiquinase USP7 as a key regulator of the proendocrine factor Ngn3 stability (Manea et al. 2023). This axis (Ngn3-USP7) represents an excellent target to improve the generation of beta cells from iPSCs. The goal of the proposed project is to understand how the axis USP7-Ngn3 is regulated during iPSCs to beta cell differentiation, to enable us to optimise the regulating pathways to improve the efficiency of beta cell generation and achieve fully functional beta cells.

To achieve this, USP7-Ngn3 inducible iPSCs lines will be generated by lentiviral infection. During the project the student will validate the different lentiviral constructs in different cell lines (including iPSCs) in a transient manner. The regulation of Ngn3 by USP7 will be evaluated by Immunoprecipitation, Immunofluorescence, qPCR and FACS. The functionality of the cells will be tested upon different USP7-NGN3 overexpression strategies.



USP7 is required for human iPSC organoids differentiation to beta cells. (A) Schematic representation of the PP to beta cell differentiation and the USP7 inhibitor regime. **(B)** Immunofluorescence for NGN3 at day 12 of differentiation (Endocrine progenitor) in organoids treated or not with USP7 inhibitor. Quantification of the % of NGN3+ cells shown in the graph **(C)** Immunofluorescence for INS/GCG/SST at day 20 of differentiation (Endocrine cells) in organoids treated or not with USP7 inhibitor. Quantification of the % of INS+ cells shown in the graph

- **References:** https://www.sancholab.org/publications

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -70-

Supervisor(s): Dr Kimberley Whitehead	E-mail (lead supervisor): kimberley.whitehead@kcl.ac.uk

Project title: Testing proof of concept that spontaneous cortical activity in human neonates is tractable

Research area (keywords): Human neurobiology, Brain development, Clinical neuroscience

Research skills (keywords): Data science, Statistics, Time series analysis, Matlab

Faculty/School/Department: Research Division of Digital Health and Applied Technologies Assessment (DHATA), Faculty of Nursing, Midwifery & Palliative Care

Location: James Clerk Maxwell Building, Waterloo Campus

Project description:

Mammalian brain development is activity-dependent. In neonatal animal models, spatio-temporally organised neural activity is required for neuronal survival and synaptic plasticity, its under- or over-expression or disruption leads to degraded cortical organisation, and its restoration is corrective. In human infants, the Whitehead Lab and others have demonstrated that cortical activity - especially in the form of bursts - may have a similar role, including after brain injury [1] (Fig). This opens the exciting possibility that such activity could be a therapeutic target, e.g. for sensory interventions [2,3].

Using an unpublished dataset of electroencephalography (EEG) recordings from human infants, you will test the proof of concept that spontaneous cortical activity could be tractable to an intervention. To do this, you will examine whether it is modified by naturally occurring variations in the sensory environment, e.g. tactile contact.

This project is a unique opportunity to engage with human neurobiology, and will solely involve data analysis (i.e. no wet lab work). Prior coding experience, or willingness to self-learn, is essential. Matlab is the most widely used programming language for EEG analysis, and there is a wealth of resources within its 'EEGLAB' infrastructure, including tutorials, toolboxes etc. For students with expertise in other software, like R or Python, it may be possible to use these in your downstream analysis pipeline. You will be expected to attend regular in-person meetings at Waterloo campus, both to share your own results-in-progress and offer constructive criticism on the work of fellow students and early career researchers.

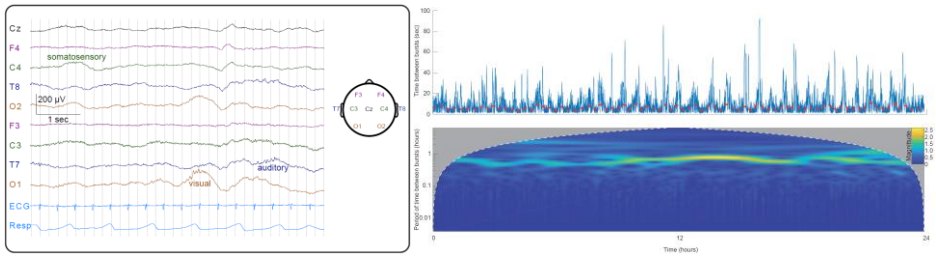


Fig. Left: Whitehead Lab, Unpublished; Right: From [1]

References: [1] Koskela, T., Meek, J., Huertas-Ceballos, A., Kendall, G. S., Whitehead, K. (2023). Clinical value of cortical bursting in preterm infants with intraventricular haemorrhage. Early Human Development. doi:10.1016/j.earlhumdev.2023.105840. [2] Whitehead, K., Rupawala, M., Laudiano-Dray, M.P., Meek, J., Olhede, S., Fabrizi, L. Posted on bioRxiv: 10.1101/2022.12.08.519675. Spontaneous activation of cortical somatosensory networks depresses their excitability in preterm human neonates. [3] Georgoulas, A., Jones, L., Laudiano-Dray, M. P., Meek, J., Fabrizi, L., & Whitehead, K. (2021). Sleep-wake regulation in pre-term and term infants. Sleep. doi:10.1093/sleep/zsaa148.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -71-

Supervisor(s): Dr Kourosh H. Ebrahimi	E-mail (lead supervisor): kourosh.ebrahimi@kcl.ac.uk

Project title: A Universal Activation Mechanism of Radical-SAM Enzymes in Humans

Research area (keywords): Immunology and Bioinorganic Chemistry

Research skills (keywords): Molecular biology, Protein biochemistry, Biophysical techniques

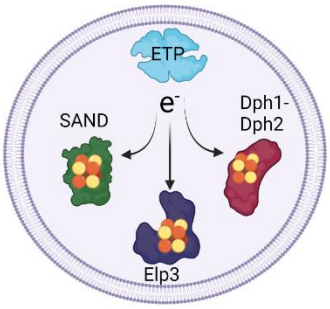
Faculty/School/Department: Faculty of Life Sciences and Medicine, Institute of Pharmaceutical Science

Location: FWB Building, Waterloo Campus

Project description:

Members of the radical S-adenosylmethionine (SAM) superfamily of enzymes are identified by a highly conserved [4Fe-4S] cluster, one of the oldest bioinorganic cofactors[1]. They are present in all life forms. All these enzymes require electrons for their activation. Several members of this superfamily are present in humans, including the antiviral enzyme SAND (also known as RSAD2 or viperin)[2], the catalytic activity of elongator complex (Elp3), and the diphthamide biosynthesis enzyme Dph1-Dph2. These enzymes play critical roles in cellular function and activation of different steps of the innate immune response.[3] The activity of these enzymes contributes to cancer cell formation and growth.[4] However, it is not clear how these proteins receive electrons to perform their catalytic function.

We have recently discovered an electron transfer partner (ETP) of SAND. Based on this finding, we propose a universal cellular ETP for all radical-SAM enzymes in the cells (Figure). In this project, we aim to test this hypothesis. You will combine molecular biology and protein biochemistry to overexpress and purify a radical-SAM enzyme and the ETP. You will then characterise both proteins using various biophysical techniques, including UV-visible spectrophotometry, NMR spectroscopy, and liquid chromatography-mass spectrometry. You will elucidate their interaction and reduction of the radical-SAM enzyme by the ETP. You will have the opportunity to visit the laboratory of one of our collaborators (Prof Simone Ciofi-Baffoni, University of Florence, Italy or Dr Peter-Leon Hagedoorn, TU Delft, the Netherlands) for one or two months. The outcomes will transform our understanding of the cellular activation mechanism of the radical-SAM enzymes. They will garner interest among scientists in various fields. Your findings are expected to contribute to a publication in a discovery journal.



References:

(1) Broderick, J. B.; Duffus, B. R.; Duschene, K. S.; Shepard, E. M. Radical S-Adenosylmethionine Enzymes. *Chem. Rev.* **2014**, *114*, 4229–4317.

(2) Ji, Y.; Li, W.; Da, A.; Stark, H.; Hagedoorn, P.-L.; Ciofi-Baffoni, S.; Cowley, S. A.; Louro, R. O.; Todorovic, S.; Mroginski, M. A.; Nicolet, Y.; Roessler, M. M.; Le Brun, N. E.; Piccioli, M.; James, W. S.; Hagen, W. R.; Ebrahimi, K. H. Radical-SAM Dependent Nucleotide Dehydratase (SAND), Rectification of the Names of an Ancient Iron-Sulfur Enzyme Using NC-IUBMB Recommendations. *Front. Mol. Biosci.* **2022**, *19*, 1032220.

(3) Ebrahimi, K. H.; Diofi, S.; Hagedoorn, P. L.; Nicolet, Y.; Le Brun, N. E.; Hagen, W. R.; Armstrong, F. A. Iron-Sulphur Clusters as Inhibitors and Catalysts of Viral Replication. *Nat. Chem.* **2022**, *14*, 253–266.

(4) Hoang, T. N.; Shahmohammadi, S.; Ebrahimi, K. H. Ancient Complexes of Iron and Sulfur Modulate Oncogenes and Oncometabolism. *Curr. Opin. Chem. Biol.* **2023**, *76*, 102338.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -72-

Supervisor(s): Dr Vincenzo Torraca	**E-mail (lead supervisor):** vincenzo.torraca@kcl.ac.uk

Project title: Investigating the bacterial factors involved in the establishment of *Shigella* persistent infections

Research area (keywords): Microbiology, Microbial genomics, Antimicrobial Resistance, Infectious Diseases, Shigella, zebrafish

Research skills (keywords): Microbiology techniques, Biosafety Level 2 working, Microbial genomics data analysis, Molecular biology techniques, in vivo infection models

Faculty/School/Department: Faculty of Life Sciences & Medicine, School of Immunology and Microbial Sciences, Department of Infectious Disease

Location: Guy's Hospital, St Thomas Street, London, SE1 9RT

Project description: *Shigella* is an important human pathogen, estimated to cause up to 165 million illnesses and 600,000 deaths yearly. There is no vaccine available that protects against shigellosis and, due to widespread antimicrobial resistance (AMR), *Shigella* is listed by the World Health Organisation as a priority pathogen.

Our lab has recently discovered that *Shigella* can establish persistent infections. Persistent infections are an important complication for public health because they are not entirely cleared by the immune system or by antibiotics (i.e., they are also antibiotic tolerant) and can become asymptomatic, which facilitates disease spreading.

In this project, we aim to identify new *Shigella* factors that contribute to persistent infections. We will first analyse bacterial genome datasets collected from patients with *Shigella* carriage. We will determine whether the persistent *Shigella* isolates carry unique factors that could be involved in persistence. The human datasets we will use consists of pairs of *Shigella* genome sequences, performed at different times during the case of persistence. Therefore, we will also assess whether the bacteria developed nucleotide changes during carriage in the host that may contribute further to efficient persistence. From these predictions, we will then select the most promising candidates and we will create *Shigella* mutants for the identified genes. Finally, we will test these mutants *in vitro* (in liquid cultures) and *in vivo* (in the zebrafish model) to confirm if these genes are involved in the establishment of antibiotic tolerance and persistent infections.

Overall, this project will offer a platform to enhance practical skills and knowledge within the field of microbiology, antimicrobial resistance, and infectious diseases, with a specific emphasis on addressing a microbial threat of global significance. An ideal student should have an interest in both laboratory microbiology and microbial genomics. However, pre-existing bioinformatics skills are not essential and will be developed in the project alongside microbiology techniques.

References:

Torraca et al., Shigella Serotypes Associated With Carriage in Humans Establish Persistent Infection in Zebrafish. J Infect Dis. 2023 Oct 18;228(8):1108-1118. doi: 10.1093/infdis/jiad326.

Bengtsson et al., Accessory Genome Dynamics and Structural Variation of *Shigella* from Persistent Infections. mBio. 2021 Apr 27;12(2):e00254-21. doi: 10.1128/mBio.00254-21.

MSc/MRes Biomedical & Molecular Sciences Research

Research Project -73-

Supervisor(s): Dr Vincenzo Abbate, Dr Asma Akter, Dr Richard Amison (if Mres)	**E-mail (lead supervisor):** vincenzo.abbate@kcl.ac.uk

Project title: Preclinical evaluation of [^{68}Ga]Ga-Schizokinen for bacterial infection imaging

Research area (keywords): Microbiology, diagnostics, Radiotracers, *In situ* infection imaging, PET/CT, ^{68}Gallium-siderophores

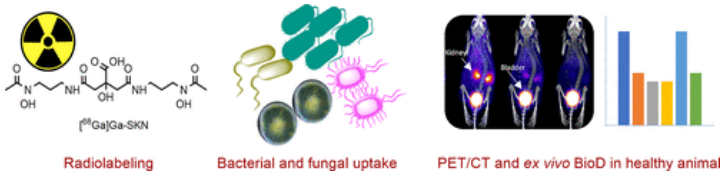
Research skills (keywords): Radiation safety, Radiolabelling, Radiotracer characterisation, Radio iTLC and HPLC, Microbiology, Cell-culture, *Galleria mellonella,* animal infection models

Faculty/School/Department: FoLSM/ Cancer & Pharmaceutical Science / AEFS

Location: Waterloo – FWB

Project description: Background: Although the use of rapid molecular and genome-based diagnostic techniques is increasing, infection-specific *in situ* imaging as a diagnostic tool remains an unmet clinical need. This approach is particularly relevant for the early detection of invasive infections in immunocompromised patients, for locating and detecting microbial infections in the body (for example, infection of implanted prosthetics), and for monitoring treatment efficacy. Total-body positron emission tomography (PET) combined with computerised tomography (CT) or single-photon emission computed tomography (SPECT) in clinical practice uses nonspecific radiotracers such as [^{18}F]fluorodeoxyglucose (FDG), [^{67}Ga]Ga-citrate, and indium-111- and technichium-99m-labelled leukocytes. None of these radiotracers can directly image the infection in the body but rather capture secondary host responses against infections.

Microbial siderophores, which transport the essential transition metal iron(III) (Fe^{3+}) into microbial cells from the surrounding environment, can be designed as bacterial- and fungal-specific radiotracers. Typically, Fe^{3+} transport is mediated by the recognition of siderophores via specific cell-surface receptors and the internalisation of siderophore-bound iron by the active transportation system of the membrane. Recently, gallium-68-labelled siderophores as PET radiotracers have gained interest for the development of *in situ* infection-specific imaging diagnostics.



At KCL, we developed a bacteria-specific radiotracer based on ^{68}Gallium-siderophore, named [^{68}Ga]Ga-Schizokinen. We have reported its radiosynthesis with ≥95% radiochemical purity, *in vitro* short-term stability, *in vitro* specificity in microbial cells that demonstrated its specificity to bacterial species, and favourable *in vivo* pharmacokinetics (PK) in a healthy animal model with Whole-body PET/CT imaging.

Aims: The proposed project will further evaluate the potential of [^{68}Ga]Ga-Schizokinen for its clinical translation. The student will aim to:

1. Confirm the long-term stability of the radiotracer in human serum and in the presence of other chelators.
2. Screen clinical strains of bacterial and fungal pathogens and determine *in vitro* toxicity in human cell line.
3. Evaluate the radiotracer in *Galleria mellonella* *in vivo* infection models with clinically relevant bacterial pathogens. (also determine *in vivo* toxicity of the radiotracers with different doses in *Galleria mellonella*)
4. Evaluate the radiotracer in *in vivo* small animal-infection model and PET/CT imaging (**If this project is funded by a Matt Wilson Scholarship and only for MRes project**).

References: (**1**) Akter A, Firth G, Darwesh AMF, et al. [^{68}Ga]Ga-Schizokinen, a Potential Radiotracer for Selective Bacterial Infection Imaging. ACS Infect Dis. 2024 Aug 9;10(8):2615-2622. doi: 10.1021/acsinfecdis.4c00067. (**2**) Akter A, Lyons O, Mehra V, Isenman H, Abbate V. Radiometal chelators for infection diagnostics. Front Nucl Med. 2023 Jan 9;2:1058388. doi: 10.3389/fnume.2022.1058388. (**3**) Ménard G, Rouillon A, Cattoir V, Donnio PY. *Galleria mellonella* as a Suitable Model of Bacterial Infection: Past, Present and Future. Front Cell Infect Microbiol. 2021 Dec 22;11:782733. doi: 10.3389/fcimb.2021.782733.

Projects 74-79

are suitable for:

MSc students

MSc Biomedical & Molecular Sciences Research

Research Project -74-

Supervisor(s): Dr Gaia Nebbia (Honorary Reader) Dr Luke Blagdon Snell (Clinical Research Fellow) Adela Alcolea-Medina (Lead Next Generation Sequencing, Clinical Scientist)	**E-mail (lead supervisor):** Gaia.Nebbia@gstt.nhs.uk
Project title: Long-read genomic sequencing of pathogens: resistance in blood borne viruses	
Research area (keywords): pathogen genomics, metagenomics, amplicon sequencing, blood borne viruses.	
Research skills (keywords): genome sequencing, nanopore, variant calling, genome assembly	
Faculty/School/Department: FOLSM, SIMS	
Location: Centre for Clinical Infectious Diseases Research, St Thomas Hospital.	

Project description:

The Centre for Clinical Infectious Diseases Research, St. Thomas' Hospital, is embedded within the infectious diseases clinical department. Our translational research aims to implement long-read pathogen sequencing as a diagnostic for infectious diseases. Namely we develop metagenomics assays as a diagnostic for causes of infection, and utilise amplicon sequencing to investigate resistance in blood borne viruses such as HIV and CMV.

Candidates will have the opportunity to develop and optimise a workflow for a specific pathogen. Workflows will involve clinical samples, and candidates will be involved at all stages of the process including sample processing, library preparation, sequencing and data analysis. Candidates will test and develop the bioinformatic analysis strategy for the long read genomic data. Target pathogens include cytomegalovirus, herpes simplex virus and hepatitis c virus. Alternatively, the project may involve whole genome sequencing of bacterial pathogens to assess for antimicrobial resistance and hospital outbreaks. Candidates will gain experience in a broad range of sequencing techniques, such as metagenomics, amplicon sequencing, and whole genome sequencing.

Our translational department has pioneered the deployment of rapid, long-read genomics for the benefit of patients. Candidates will work closely with the wider team, including clinicians, clinical scientists, bioinformaticians and researchers. Our team has deployed various pathogen sequencing assays into clinical setting, for agnostic diagnostics of pneumonia and for specific pathogens such as SARS-CoV-2, and influenza.

References:

1. Charalampous, T., Alcolea-Medina, A., Snell, L.B. *et al.* Evaluating the potential for respiratory metagenomics to improve treatment of secondary infection and detection of nosocomial transmission on expanded COVID-19 intensive care units. *Genome Med* **13**, 182 (2021). https://doi.org/10.1186/s13073-021-00991-y

2. Snell LB, *et al*. Real-Time Whole Genome Sequencing to Guide Patient-Tailored Therapy of Severe Acute Respiratory Syndrome Coronavirus 2 Infection. Clin Infect Dis. 2023 Mar 21;76(6):1125-1128. doi: 10.1093/cid/ciac864. PMID: 36327795; PMCID: PMC10029986.

3. Alcolea-Medina A, Snell LB *et al* The ongoing Streptococcus pyogenes (Group A Streptococcus) outbreak in London, United Kingdom, in December 2022: a molecular epidemiology study. Clin Microbiol Infect. 2023 Jul;29(7):887-890. doi: 10.1016/j.cmi.2023.03.001. Epub 2023 Mar 15. PMID: 36925107; PMCID: PMC10769882.

MSc Biomedical & Molecular Sciences Research Project

Research Project -75-

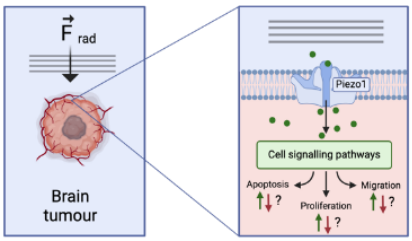
Supervisor(s): Dr. Antonios Pouliopoulos and Lauren Gomes	**E-mail (lead supervisor):** antonios.pouliopoulos@kcl.ac.uk
Project title: Investigating the role of Piezo1 in brain cancer cells following focused ultrasound exposure	
Research area (keywords): Molecular biology, Ultrasound, Biomedical engineering	
Research skills (keywords): Cell culture, siRNA, Focused ultrasound, Immunostaining, qPCR	
Faculty/School/Department: Department of Surgical & Interventional Engineering, School of Biomedical Engineering and Imaging Science	
Location: 4th floor Lambeth Wing, St. Thomas Hospital	

Project description:

Background: Glioblastoma multiforme (GBM), an aggressive brain tumour, exhibits altered mechanobiology including increased expression of mechanosensitive ion channel Piezo1 (Chen et al., 2018; Qu et al., 2020) which is associated with poor prognosis. Focused ultrasound (FUS) is being explored as a new treatment modality in neuro-oncology (Meng et al., 2021; Pouliopoulos et al., 2021; Wei et al., 2021). However, the direct bioeffects of primary acoustic radiation forces on GBM and diffuse midline glioma (DMG) cells are not well characterised (**Figure 1**).

Aims: We aim to explore the role of Piezo1 in brain tumour cells by knocking down its gene expression and developing an immunostaining protocol to image migration and adhesion markers after Piezo1 knockdown and/or FUS exposure.

Methods: The student will perform cell culture, siRNA gene knockdown, qPCRs, and develop an immunostaining protocol.



Expected outcomes: By the end of the project, the student will have performed various molecular biology techniques and will have developed an immunostaining protocol to help elucidate the role of Piezo1 in brain cancer cells. The student will get opportunities to assist with FUS experiments.

Figure 1. The direct bioeffects of FUS on brain tumours (DMG/GBM), including cell signalling pathways downstream of Piezo1 ion channel activation, are not well characterised.

References:

Chen, X., Wanggou, S., Bodalia, A., Zhu, M., Dong, W., Fan, J.J., et al. (2018). A Feedforward Mechanism Mediated by Mechanosensitive Ion Channel PIEZO1 and Tissue Mechanics Promotes Glioma Aggression. Neuron *100*: 799-815.e7.

Meng, Y., Hynynen, K., and Lipsman, N. (2021). Applications of focused ultrasound in the brain: from thermoablation to drug delivery. Nat. Rev. Neurol. 17: 7–22.

Pouliopoulos, A. N., Kwon, N., Jensen, G., Meaney, A., Niimi, Y., Burgess, M. T., Ji, R., et al. (2021). Safety evaluation of a clinical focused ultrasound system for neuronavigation guided blood-brain barrier opening in non-human primates. Scientific reports, 11(1), 15043.

Qu, S., Li, S., and Hu, Z. (2020). Upregulation of piezo1 is a novel prognostic indicator in glioma patients. Cancer Manag. Res. *12*: 3527–3536.

Wei, H. J., Upadhyayula, P. S., Pouliopoulos, A. N., Englander, Z. K., Zhang, X., Jan, C. I., Guo, J., et al. (2021). Focused Ultrasound-Mediated Blood-Brain Barrier Opening Increases Delivery and Efficacy of Etoposide for Glioblastoma Treatment. International journal of radiation oncology, biology, physics, 110(2), 539–550.

MSc Biomedical & Molecular Sciences Research

Research Project -76-

Supervisor(s): Dr. Lei Lu	**E-mail (lead supervisor):** lei.lu@kcl.ac.uk

Project title: Deep Generative Model for Cardiac Signal Captioning in Cardiovascular Disease Diagnosis

Research area (keywords): Deep learning, generative AI, cardiovascular disease

Research skills (keywords): Deep learning

Faculty/School/Department: School of Life Course & Population Sciences

Location: King's College London, Guy's Campus, Great Maze Pond, London SE1 1UL

Project description:

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, underscoring the critical importance of accurate and timely diagnosis. Cardiac signals, particularly the electrocardiogram (ECG), are fundamental tools in the screening and diagnosis of CVDs. Traditionally, the interpretation of these signals relies on expert cardiologists who employ rule-based methods to analyse the data. However, even for seasoned professionals, interpreting ECGs is a time-consuming and complex task, often subject to variability both between different physicians and within the same physician over time. This variability can lead to inconsistencies in diagnosis, potentially affecting patient outcomes.

Recent advancements in deep learning have demonstrated the potential of large language models (LLMs) in the fields of knowledge representation and automated information generation. Preliminary research has begun to explore the use of LLMs for generating diagnostic reports based on ECG data. For instance, initial studies have utilised time and frequency domain features as inputs for generating these reports, rather than directly using ECG morphological features, which are more closely related to the underlying cardiac conditions. This project seeks to advance this area of research by developing a deep generative model specifically designed for cardiac signal captioning, with a focus on generating precise and clinically relevant medical reports that can aid in the diagnosis of cardiovascular diseases. The project will leverage the PTB-XL dataset, a large and publicly available dataset of ECG records, to train and evaluate the proposed model. The development and implementation of this model will be supported by the team's expertise in clinical machine learning, ensuring that the approach is both technically robust and clinically meaningful. Through this work, we aim to contribute to the development of automated tools that can assist cardiologists in the accurate and efficient interpretation of cardiac signals, ultimately improving the diagnosis and management of CVDs.

References:

[1] Qiu, Jielin, et al. Transfer knowledge from natural language to electrocardiography: Can we detect cardiovascular disease through language models?. arXiv preprint arXiv:2301.09017 (2023).

[2] Wagner, Patrick, et al. PTB-XL, a large publicly available electrocardiography dataset. Scientific Data 7.1 (2020): 1-15.

MSc Biomedical & Molecular Sciences Research

Research Project -77-

Supervisor(s): Dr Cynthia Yu-Wai-Man	**E-mail (lead supervisor):** cynthia.yu-wai-man@kcl.ac.uk

Project title: Development of a breakthrough anti-fibrotic gene therapy to improve surgical outcomes and reduce re-admission rates for patients with severe glaucoma.

Research area (keywords): Gene therapy, RNA therapeutics

Research skills (keywords): Gene therapy, Transfection, Molecular Biology

Faculty/School/Department: Faculty of Life Sciences & Medicine

Location: Glaucoma & Therapeutics Lab, Hodgkin Building, King's College London

Project description:

Glaucoma is the leading cause of irreversible blindness and affects 76 million people worldwide. Glaucoma filtration surgery is the mainstay of surgical treatment in glaucoma, but the failure rate is high at 50% after 5 years. This project focuses on the pre-clinical development of a novel targeted gene therapy to increase the surgical success in glaucoma by reducing post-surgical scarring, without exposure to cytotoxic drugs like mitomycin-C.

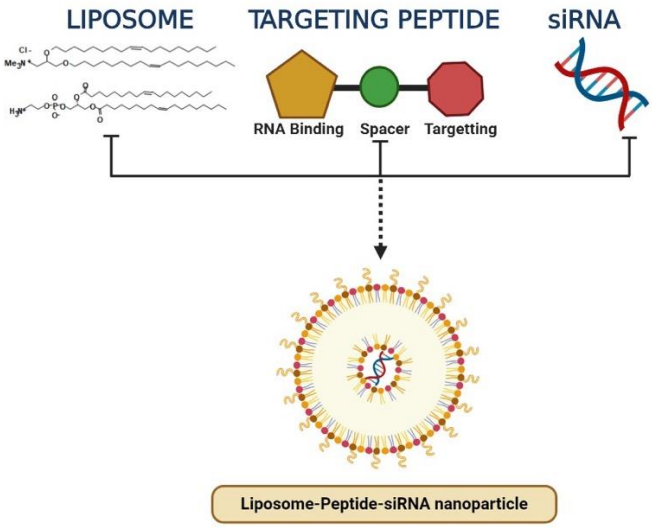
Our lab has developed innovative lipid and peptide-based nanoparticle encapsulation methods, which protect RNA molecules from degradation and deliver them directly to the affected site for targeted silencing of pathogenic genes [1, 2].

We aim to rapidly progress the development of the gene therapy and conduct toxicology/efficacy studies to provide vital data for future human trials.

Project objectives:

1) The student will optimise a tailored mix of peptides/lipids encapsulating a genetic sequence enabling active targeted uptake in human conjunctival fibroblasts. Skills learned: Gene therapy, transfection, molecular biology

2) The student will develop a toxicological report, detailing the *in vivo* efficacy and toxicological profile of the optimised RNA therapeutics in the rabbit model of glaucoma surgery. Skills learned: Examining animals, histology, fluid sampling for high-performance liquid chromatography.



References:

1. Fernando O, Tagalakis AD, Awwad S, Brocchini S, Khaw PT, Hart SL, **Yu-Wai-Man C**. Development of targeted siRNA nanocomplexes to prevent fibrosis in experimental glaucoma filtration surgery. *Mol Ther*. 2018 Dec; 26(12): 2812-2822.
2. Sanghani A, Kafetzis KN, Sato Y, Elboraie S, Fajardo-Sanchez J, Harashima H, Tagalakis AD, **Yu-Wai-Man C**. Novel PEGylated Lipid Nanoparticles Have a High Encapsulation Efficiency and Effectively Deliver MRTF-B siRNA in Conjunctival Fibroblasts. Pharmaceutics. 2021; 13(3): 382.

MSc Biomedical & Molecular Sciences Research

Research Project -78-

Supervisor(s): Dr Cynthia Yu-Wai-Man	**E-mail (lead supervisor):** cynthia.yu-wai-man@kcl.ac.uk

Project title: Developing a novel 3D-printed drug implant in minimally invasive glaucoma surgery

Research area (keywords): Drug delivery, 3D printing, Drug implant

Research skills (keywords): Drug formulation, Drug release, Cell biology, Molecular biology

Faculty/School/Department: Faculty of Life Sciences & Medicine

Location: Glaucoma & Therapeutics Lab, Hodgkin Building, King's College London

Project description:

Glaucoma is the leading cause of irreversible blindness and affects 76 million people worldwide. Over the last decade, the use of minimally invasive glaucoma surgery (MIGS) devices, such as iStent, Hydrus, Kahook dual blade, have increased exponentially; these devices are inserted into Schlemm's canal or the suprachoroidal space to increase the drainage of aqueous fluid out of the eye and thus to lower the intraocular pressure.

However, MIGS devices have a high failure rate of over 50% after 1 year due to scarring, and fibrosis is the critical determinant of the long-term success in MIGS devices. The trabecular meshwork (TM) becomes scarred and aqueous fluid can no longer drain out of the eye, leading to increased intraocular pressure and optic nerve damage.

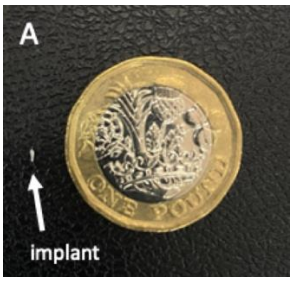
Specific pro-inflammatory cytokines in the aqueous fluid, e.g. TGF-beta and IL-6, increase the risk of scarring in MIGS devices, and sustained and targeted inhibition of this cytokine signature is an effective and safe therapeutic modality to prevent scarring and to increase the success rate in MIGS devices [1].

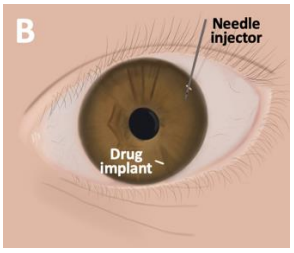
Project objectives:

1) The student will develop a biocompatible sustained-release 3D-printed drug implant containing polymer solutions and using crosslinking or thermogelling with the new drug [2].

2) The student will test the percentage and duration of release of the drug as the polymer degrades over time. The student will measure the effects of the drug polymer matrix on key fibrotic genes, such as *ACTA2, CTGF, MRTF-B*, in human TM cells using real-time qPCR.

3) The student will also assess the cytotoxicity of the drug polymer matrix on human TM cells using cell viability assays. The most efficient drug polymer matrix will be based on the highest silencing efficiency of key fibrotic genes, a gradual drug release over several weeks/ months, stable storage conditions, and low cytotoxicity.





References:

1. **Yu-Wai-Man C**, Tagalakis AD, Meng JH, Bouremel Y, Lee RM, Virasami A, Hart SL, Khaw PT. Genotype-phenotype associations of IL-6 and PRG4 with conjunctival fibrosis after glaucoma surgery. JAMA Ophthalmol. 2017; 135(11): 1147-1155.
2. Tan G, Ioannou N, Mathew E, Tagalakis AD, Lamprou DA, **Yu-Wai-Man C**. 3D printing in Ophthalmology: From medical implants to personalised medicine. Int J Pharm. 2022; 625: 122094.

MSc Biomedical & Molecular Sciences Research

Research Project -79-

Supervisor(s): Dr James Bowe, Dr Kim Jonas	**E-mail (lead supervisor):** james.bowe@kcl.ac.uk kim.jonas@kcl.ac.uk

Project title: Effects of Type 1 Diabetes on markers of Polycystic Ovary Syndrome

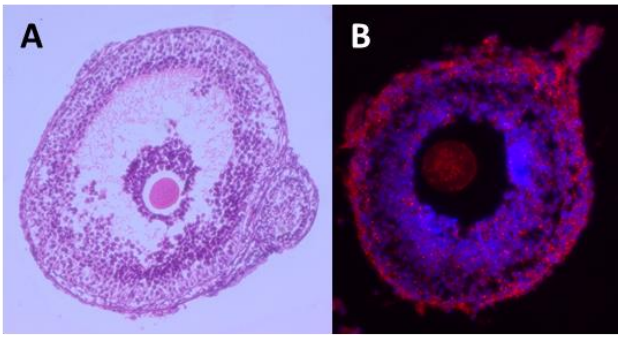
Research area (keywords): Type 1 Diabetes, Polycystic Ovary Syndrome, Pancreatic Beta-cells, Ovarian follicles

Research skills (keywords): Tissue histology, Immunostaining, Image analysis, qPCR

Faculty/School/Department: School of Cardiovascular and Metabolic Medicine & Sciences

Location: Guy's Campus, Hodgkin Building

Project description:



Representative images showing haemotoxylin & eosin (A) and immunostaining (B) of ovarian follicles.

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 6-15% worldwide. Although the aetiology of PCOS is unknown, the mechanism that PCOS develops has been linked to altered levels of the pituitary hormones LH and FSH, leading to dysfunctional ovarian follicle development and elevated androgen levels. This results in reduced fertility and a range of additional adverse effects including obesity and Type 2 Diabetes (T2D); elevated androgens lead to insulin resistance and hyperinsulinaemia, contributing to the development of T2D, whilst high insulin levels enhance androgen synthesis in the ovaries contributing to PCOS. This harmful interaction between T2D and PCOS has been well-characterised in previous studies.

It is perhaps less appreciated that approximately 25% of women with Type 1 Diabetes (T1D) also develop PCOS, a prevalence considerably higher than the general population. T1D is characterised by autoimmune destruction of the insulin-producing beta-cells of the pancreas. While insulin resistance and hyperinsulinaemia drives the interaction between T2D and PCOS, individuals with T1D classically have normal insulin sensitivity but an inability to produce insulin. Thus, the association between T1D and PCOS is likely to involve distinct pathophysiological mechanisms, though these are poorly understood. This study aims to assess markers of PCOS in animal models of T1D and examine whether different animal models of T1D show signs of developing PCOS ovaries. This is a collaborative project between Dr James Bowe who has a background in animal models of diabetes and Dr Kim Jonas who has a background in ovarian physiology. Once we have characterised these T1D animal models, the long-term aims of the project would be to look into the mechanisms linking T1D and PCOS symptoms.

For this project the student will work closely with experienced members of both labs. Female streptozotocin-administered mice will be used as a model of T1D alongside non-diabetic controls. Glucose control will be assessed through monitoring blood glucose levels and plasma insulin levels, alongside assessment of reproductive cycles and levels of reproductive hormones. Ovarian samples taken from these mouse models will be analysed using histological and immunostaining techniques to measure the number and morphology of the ovarian follicles.

References: Johnson GP, Onabanjo CGA, Hardy K, Butnev VY, Bousfield GR, Jonas KC (2022) Follicle-Stimulating Hormone Glycosylation Variants Distinctly Modulate Pre-antral Follicle Growth and Survival. *Endocrinology* **163(12);** DOI: 10.1210/endocr/bqac161

Projects 80-92

are suitable for:

MSc students

MRes students

MSci Molecular Genetics students

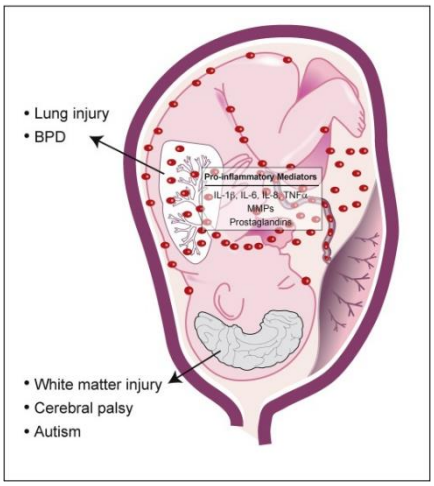
MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -80-

Supervisor(s): **Dr Ashley Boyle**	**E-mail (lead supervisor):** ashley.boyle@kcl.ac.uk
Project title: Novel treatments for preterm birth	
Research area (keywords): Reproductive Biology, Immunology, Gene Therapy	
Research skills (keywords): DNA/RNA extraction, qPCR, cell culture	
Faculty/School/Department: Department for Women and Children's Health, FoLSM	
Location: St Thomas' Hospital	

Project description:



Preterm birth (delivery before 37 weeks) is a significant global health issue, accounting for approximately 11% of births worldwide and is the leading cause of morbidity and mortality in babies. It is commonly associated with microbes, such as bacteria. Currently, treatments are limited; antibiotics are ineffective. We have successfully mimicked preterm birth and neonatal brain damage in pregnant mice using a bioluminescent strain of *E. coli*, which allows us to track the infection.

Defects in cervical immunity may be a key feature of preterm birth. The cervix, a physical and immunological barrier, protects the uterus and fetus. The cervical mucus plug, a latticed structure containing immune cells and antimicrobial peptides, plays an important role in preventing bacterial uterine invasion.

In this project, we aim to investigate the ability of an antimicrobial gene therapy to protect pup development. This will involve assessing pup brains, lungs and/or gut for signs of inflammation or damage. We will also analyse the maternal reproductive tissues for evidence of inflammation.

The student will be allowed some flexibility when choosing which specific tissue to analyse. There is also scope to test gene therapies on human cervical cells (cell culture).

References:

Boyle et el. Preterm birth: Inflammation, fetal injury and treatment strategies. J Reprod Immunol. 2017 Feb;119:62-66. doi: 10.1016/j.jri.2016.11.008.

Boyle A. K., Tetorou K., Suff N., Beecroft L., Mazzaschi M., Hristova M., Waddington S.N., Peebles D. Ascending vaginal infection in mice induces preterm birth and neonatal morbidity. bioRxiv 2023.08.14.553220; doi:10.1101/2023.08.14.553220.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -81-

Supervisor(s): Prof. Cynthia Andoniadou Dr Yasmine Kemkem (Senior Postdoctoral Research Associate)	**E-mail (lead supervisor):** cynthia.andoniadou@kcl.ac.uk
Project title: The role of succinate metabolism in the regulation of multipotent neural crest progenitors	
Research area (keywords): developmental biology, genetics	
Research skills (keywords): mouse genetics, dissection, histology, molecular biology, microscopy	
Faculty/School/Department: Dentistry, Oral & Craniofacial Sciences (FoDOCS)	
Location: Floor 27, Tower Wing, Guy's Campus	

Project description:

Mutations in genes encoding subunits of the succinate complex in humans (*SDHx*), can drive the formation of tumours in many tissues. When succinate accumulates, mutant cells experience 'pseudohypoxia', and activate pathways associated with regulating cell behaviour, including cell division. Our group study a group of tumours called PPGL, which can be located along the neural crest (NC) migration route. These tumours are derived from progenitors of the NC lineage, which are specified during embryonic development. We have an interest in *SDHB*, as mutations in this gene lead to the most aggressive tumours.

In order to determine SDHB function in the NC, we generated mice in which *Sdhb* is specifically deleted in the NC cells, using the Cre-loxp system. We used an early Cre driver to delete *Sdhb* in NC as soon as these are specified (Wnt1-Cre), and a Cre driver deleting *Sdhb* in NC cells only upon tamoxifen injection (Sox10-CreERT2).

Our preliminary data demonstrate that early deletion of *Sdhb* in the murine NC leads to a dramatic absence of NC-derived structures during embryonic development e.g. part of the face.

This project will investigate:

1. The time window when SDHB is required for normal NC progenitor specification/function.
2. How NC progenitor behaviour is affected following *Sdhb* deletion.
3. If *Sdhb* deletion at later stages results in the same phenotype or leads to tumours.

The student will perform a thorough phenotypic characterisation at embryonic stages, to fully characterise the effect on the neural crest and the impact on neural crest-derived structures (e.g. the adrenal medulla). They will learn how to dissect embryos, prepare them for sectioning and histological analyses, conduct immunofluorescence staining and mRNA in situ hybridisation, document and analyse results using brightfield and confocal microscopy, and formulate interpretations. They will also be expected to become involved in genotyping the mouse colony through PCRs, support in planning and setting up new experiments and contribute to the analysis as the project develops.

References: Scriba et al. PMID: 32158431 , Goncalves et al. PMID: 34127497, Santambrogio et al. bioRxiv doi.org/10.1101/2023.10.28.564519 .

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -82-

Supervisor(s): Dr Antonio de Marvao; Maternal Cardiovascular Medicine & Genomics	**E-mail (lead supervisor):** antonio.de_marvao@kcl.ac.uk

Project title: The genomic determinants of outcomes of pregnancy

Research area (keywords): genomics; genetics; pregnancy; preeclampsia; cardiovascular; maternal cardiology; ancestry; hypertension

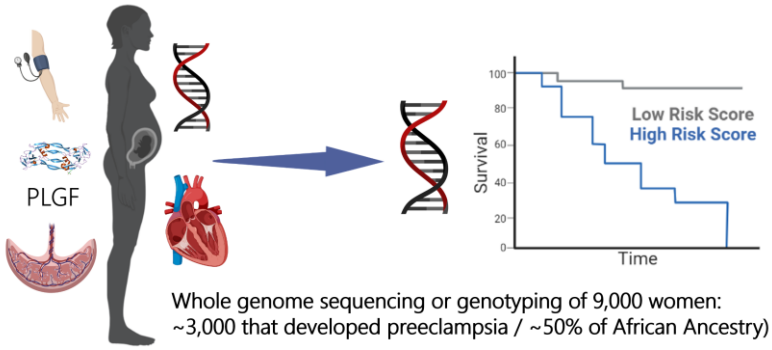
Research skills (keywords): bioinformatics; statistics; GWAS; Computational data analysis

Faculty/School/Department: Department of Women and Children's Health & School of Cardiovascular and Metabolic Medicine and Sciences King's College London

Location: Fetal Medicine Foundation, Denmark Hill and / or St Thomas' Hospital campus

Background: High blood pressure affects approximately 1 in 10 pregnant women in the UK. Common hypertensive disorders of pregnancy include gestational hypertension (8% of pregnancies) and preeclampsia (2-3% of pregnancies). Recent genetic studies have started to provide evidence of a polygenic component in preeclampsia.[1] Women of African ancestry are at highest risk for preeclampsia (2.5 times). However, existing studies investigating genetic determinants of preeclampsia include predominantly or exclusively women of European ancestry (78% White-Europeans; 2% African).

Project: We aim to evaluate how genomics can help improve prediction of maternal and fetal pregnancy outcomes, such as preeclampsia, birth weight and preterm birth. We have genotyped 9,000 women of the 150,000 women that were recruited into the Fetal Medicine Foundation biobank during their pregnancies. Of these, 3,000 developed preeclampsia and half are of African Ancestry. All participants have had deep maternal and fetal phenotyping, including maternal demographics, biomarkers and ultrasound doppler assessment of placental blood flow.



Whole genome sequencing or genotyping of 9,000 women:
~3,000 that developed preeclampsia / ~50% of African Ancestry)

Skills: This will be a bioinformatics project where the student will learn how to perform genome-wide association studies and / or rare variant analysis to determine the maternal and fetal genomic determinants of outcomes of pregnancy, with a specific focus on developing improved polygenic scores for women of African ancestry.

References: 1 - Honigberg, M.C., et al. Polygenic prediction of preeclampsia and gestational hypertension. Nat Med 29, 1540–1549 (2023). https://doi.org/10.1038/s41591-023-02374-9

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -83-

Supervisor(s): Dr Joseph Wanford	**E-mail (lead supervisor):** joseph.wanford@kcl.ac.uk

Project title: An ON/OFF Genetic Virulence Switch in Antibiotic-Resistant *Klebsiella pneumoniae*

Research area (keywords): Infectious Diseases, Microbiology, Innate Immunity, Genomics

Research skills (keywords): Molecular genetics, mammalian cell culture, infection

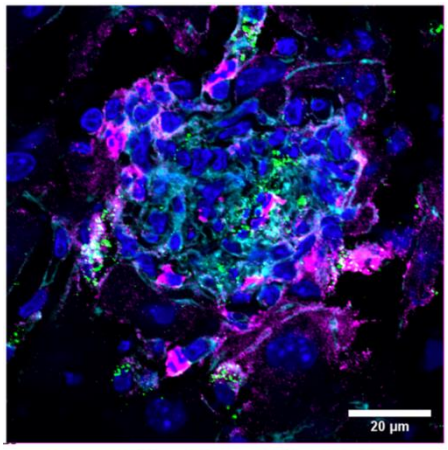
Faculty/School/Department: School of Immunology and Microbial Sciences

Location: Guy's Hospital, 2nd Floor Borough Wing

Project description:

The Wanford group are interested in how multi-drug resistant pathogens - including the WHO priority pathogen *Klebsiella pneumoniae* - cause disease. *K. pneumoniae* cause invasive infections with high mortality, and their major virulence factors is a thick polysaccharide capsule. We have identified a genetic switch for virulence – termed phase variation – which reversibly inactivates capsule synthesis. This switch is mediated by slipped strand mispairing mutations in genes encoding regulators of the capsule and enables the pathogen to adapt to rapidly changing environments in the host (see references on 'phase variation'). Our preliminary data indicate that this genetic switch generates multiple sub-populations of extra- and intracellular bacteria during infection which undermines treatment with antibiotics.

To extend these observations, this project will involve (1) bioinformatic characterisation of the phase mechanism in hypervirulent strains of *Klebsiella* by comparative genomics, (2) molecular genetics analysis of phase variation events *in vitro* using clinical isolates, and (3) analysis of the dynamics of capsule phase variation when exposed to the human innate immune system. The successful candidate can choose which of these 3 streams they are more interested in exploring. The successful candidate will contribute data to a manuscript in preparation.



References:

Wanford, Joseph J., et al. "Interaction of Klebsiella pneumoniae with tissue macrophages in a mouse infection model and ex-vivo pig organ perfusions: an exploratory investigation." The Lancet Microbe 2.12 (2021): e695-e703.

Wanford, Joseph J., et al. "Meningococcal core and accessory phasomes vary by clonal complex." Microbial Genomics 6.5 (2020).

Bayliss, Christopher D., and Michael E. Palmer. "Evolution of simple sequence repeat–mediated phase variation in bacterial genomes." Annals of the New York Academy of Sciences 1267.1 (2012): 39-44

Figure 1. Hypervirulent *Kp* infection of the liver. Capsular polysaccharide prevents killing by tissue macrophages and drives uncontrolled inflammation.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -84-

Supervisor(s): Norah Fogarty	**E-mail (lead supervisor):** norah.fogarty@kcl.ac.uk

Project title: Examination of the role of GATA2 and GATA3 in human trophoblast stem cells

Research area (keywords): placenta, stem cells, programming

Research skills (keywords): molecular biology, immunofluorescence imaging, cellular programming

Faculty/School/Department: Faculty of life sciences and medicine/BMBS/Centre for Gene Therapy and Regenerative Medicine

Location: 28th floor, Tower Wing, Guy's hospital

Project description:

The correct functioning of the human placenta is crucial to ensure a healthy pregnancy outcome. However, despite this critical role, it is one of the least understood organs. 5 days after fertilisation the human embryo forms a blastocyst comprised of an outer layer of trophectoderm (TE), that gives rise to trophoblast cells (TB) in the placenta. After implantation the TE differentiates into cytotrophoblast cells (CTBs) which give rise to the multinucleated syncytiotrophoblast (STB) and extravillous trophoblast cells (EVTs) that invade the maternal decidua. A conserved molecular cascade regulates TE initiation in cow, mouse and human [1], but subsequent TE development is poorly defined in human due to a historical lack of *in vitro* models for molecular analysis. Recently derived human trophoblast stem cells now provide us a platform for trophoblast research.

Our lab has identified 5 transcription factors, including GATA2 and GATA3, that when overexpressed in human embryonic stem cells transdifferentiates them into induced trophoblast stem cells [2]. This implicates these factors as key regulators of human trophoblast identity. The GATA transcription factor family are DNA binding proteins that are capable of activating and repressing target gene expression. Intriguingly, in the mouse there is redundancy between GATA2 and GATA3 whereby loss of both of the factors but not of either one alone results in embryonic lethality [3]. Moreover, GATA3 single factor overexpression in mouse embryonic stem cells transdifferentiates them to mouse TSCs. This in contrast in the human, where we observed that both GATA2 and GATA3 are required to establish trophoblast identity.

The goal of this project is to identify the role of GATA2 and GATA3 in human trophoblast stem cell regulation. The student will perform CUT&Tag (Cleavage Under Targets and Tagmentation) on human TSCs to investigate regions of the genome directly bound by GATA2. We will compare this with previously generated data for GATA3. This analysis will identity targets common to both transcription factors as well as those bound uniquely by each factors. The student will then use gene knockout techniques to deplete GATA2 and GATA3 in hTSCs. Molecular and imaging techniques including immunofluorescence analysis and RT-qPCR will be used to profile resultant cells and determine the phenotypic effect of loss of transcription factors. This work will provide a comprehensive analysis of the role of GATA factors in the transcriptional network of human trophoblast.

Techniques: Cell culture, CUT&Tag, CRISPR-Cas9 genome editing, immunofluorescence analysis, RT-qPCR

References:

[1] Gerri et al. (2020) https://doi.org/10.1038/s41586-020-2759-x
[2] Balestrini et al. (Accepted, Development)
https://www.biorxiv.org/content/biorxiv/early/2024/03/11/2021.08.18.456785.full.pdf
[3] Kidder and Palmer et al. (2010) DOI: 10.1101/gr.101469.109

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -85-

Supervisor(s): David Morris	**E-mail(lead supervisor):**david.l.morris@kcl.ac.uk

Title: Determining the causal genes and disease relevant tissues behind the genetics of Lupus.

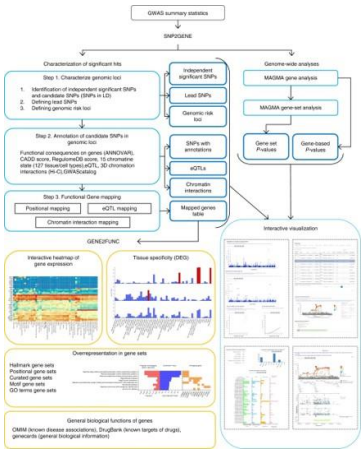
Research area (keywords): Genetics, Complex Disease, Lupus.

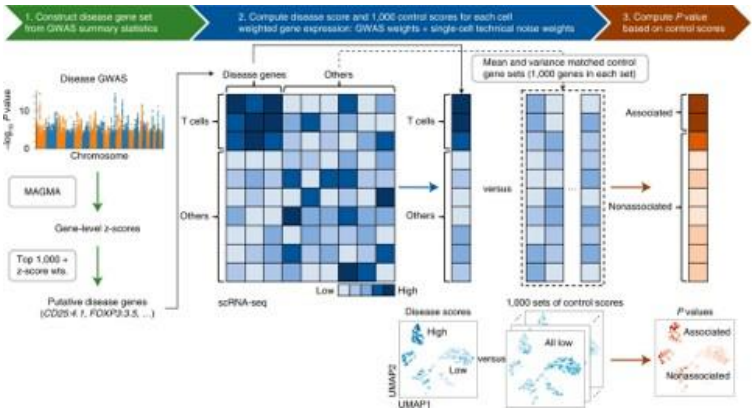
Research skills (keywords): Genetics, Statistics, Bioinformatics.

Faculty/School/Department: Life Sciences and Medicine, Medical and Molecular Genetics

Location: Guys tower Wing, Guys Campus

Project description: Systemic Lupus Erythematosus (SLE, Lupus) is a complex autoimmune disease with a relatively high heritability. There are over 100 loci published as associated with SLE[1], yet in most we do not know the causal variants(s), genes and in which cell types they exert their effects. The published associated variants are unlikely to be causal, as they are most likely correlated with the true causal variants. It is difficult to identify true causal variants, and therefore infer the functional consequences, from genetic association studies alone because sample sizes are not large enough to give the statistical power to differentiate between multiple competing SNPs with similar evidence for association. We can improve on this by increasing sample size, but this will only help reduce the number of competing SNPs in most cases. What is required to fully identify the true causal variants and explain their role in the disease is additional data that informs us of the function of these variants from genotype to molecular activity such as gene and protein expression. This project will use the most extensive collection of genetic association data on SLE across multiple populations together with functional data with the aim to identify the causal genes and relevant cell types to explain the processes from genetic variation to functional variation. The genetic association data will be used in combination with programmes such as MAGMA[2] and FUMA[3], noted below, to make inference on which are the most likely genes, cell types and functional consequences. The scope of this project is dependent on the student's interest, ranging from the use of summary association data with MAGMA and FUMA to create a pipeline for analyses and interpretation of results using these approaches, to more detailed analyses of genetic loci by, for example, running genetic association analyses and comparing directly to gene expression (eQTL) data, determining whether signals overlap between disease and gene-expression association (Co-localization analyses), and downloading publicly available genomic data to overlay on these results to further explain the associations and pinpoint likely causal polymorphisms. The student will learn statistical methodology for use in genetic studies of disease, programming languages such as R and how to use software for analysis. The **left figure below** shows the use of FUMA[3] where GWAS summary data is used to infer genomic effects, gene expression regulation and genes involved in the disease, the **right figure below** shows the use of MAGMA to determine relevant genes and cell types.





References: [1]PMID 33536424 [2] PMID 36050550, [3] PMID 29184056

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -86-

Supervisor(s): Dr Panicos Shangaris, Dr Joanna Jackow, Dr Ciro Chiappini	**E-mail (lead supervisor):** panicos.shangaris@kcl.ac.uk

Project title: In Utero Treatment of Sickle Cell Disease (SCD) Using Nanoparticle-Mediated Gene Editing

Research area (keywords): Gene Editing, Nanoparticle Delivery System, Prenatal Therapy, Immunology,

Research skills (keywords): Gene Editing, Prenatal Nanoparticles, Transplantation, Prenatal Therapy

Faculty/School/Department: Faculty of Life Sciences and Medicine, School of Immunology & Microbial Sciences

Location: Immunoregulation Laboratory, 5th Floor, Guy's Hospital (Dr Shangaris) and the Institute of Dermatology 9th floor Guy's Hospital (Dr Jackow),

Sickle Cell Disease (SCD) is a severe, progressively debilitating, and life-threatening genetic disorder characterized by frequent vaso-occlusive episodes and chronic anaemia, leading to significant morbidity, including chronic organ damage, infections, and strokes in both children and adults. It is a leading cause of mortality in Africa, contributing to 9-15% of all child deaths, with projections indicating an increase to 400,000 new births annually by 2050. The condition significantly reduces the quality of life and often shortens lifespan, with most available treatments focusing only on symptom management rather than a cure. The lifetime cost of managing SCD in the United States is estimated at $460,000, with the UK NHS incurring £18 million in SCD-related admissions during 2010-2011. Approximately 4,000 SCD patients are currently under the care of King's Health Partners, representing nearly one-third of the UK's total SCD patient population. Given the high burden of SCD, developing a curative treatment, particularly for fetuses diagnosed with SCD, holds substantial promise for both improving patient outcomes and reducing healthcare costs. The advent of non-invasive prenatal diagnosis (NIPD) through the analysis of circulating fetal DNA (cfDNA) allows for the early detection of SCD and other congenital conditions as early as ten weeks into gestation. Presently, the options available to parents following a prenatal SCD diagnosis are limited to pregnancy termination or vigilant monitoring followed by postnatal treatment. This proposal introduces a potential third option: in utero treatment of SCD through gene correction facilitated by nanoparticles designed to deliver gene-editing tools.

Research Background and Progress:

Previous research by our team has successfully developed and optimized gene-editing tools, notably CRISPR/Cas9 and base editors like ABE8e-NRCH, aimed at correcting the E6V mutation in the HBB gene responsible for SCD. In particular, the application of CRISPR/Cas9 demonstrated effective disruption of the target gene in sickle cells derived from human umbilical cord blood (sHUDEP-2 cells). Furthermore, the ABE8e-NRCH base editor achieved complete correction of the SCD mutation, converting the pathogenic GTG codon back to its non-pathogenic form.

Project Aims:

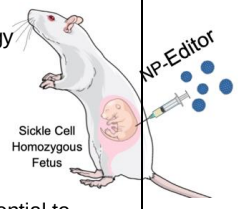
The next phase of our research will focus on encapsulating these gene-editing tools within bioengineered nanoparticles and testing their efficacy in vitro. The project will involve the following steps:

1. **Nanoparticle Formulation and Characterization:** Collaborating with Dr. Jackow and Dr. Chiappini, the student will assist in encapsulating gene-editing tools within lipid nanoparticles (LNPs). The LNPs will be characterized for size, polydispersity index, encapsulation efficiency, and morphology using techniques such as transmission electron microscopy (TEM) and dynamic light scattering.
2. **In Vitro Testing:** The student will test the biocompatibility, cellular uptake, and targeting efficiency of the nanoparticles in human fetal liver cells and erythroid cell lines (HUDEP SS and wild type). This will involve evaluating cellular internalization using confocal microscopy, PCR, and flow cytometry. The viability and functionality of hematopoietic stem cells (HSCs) post-nanoparticle treatment will also be assessed.
3. **Data Analysis and In Vivo Potential:** Under the supervision of Dr. Shangaris, the student will analyze the in vitro data and contribute to planning potential in vivo experiments. These experiments would involve testing the nanoparticles in relevant animal models to assess the feasibility of in utero gene correction.

Skills and Techniques:

During the course of this 6-month project, the student will gain hands-on experience with a range of molecular biology techniques, including:

- Nanoparticle formulation and characterization
- In vitro culture and transfection of HSCs
- Flow cytometry and confocal microscopy for cellular analysis
- Statistical analysis of experimental data



This project represents an important step towards the development of a prenatal gene therapy for SCD, with the potential to prevent the birth of children affected by this devastating condition

References: Cortabarria AS de V, Makhoul L, Strouboulis J, Lombardi G, Oteng-Ntim E, Shangaris P. In utero Therapy for the Treatment of Sickle Cell Disease: Taking Advantage of the Fetal Immune System. Frontiers in Cell and Developmental Biology. 2021 Jan 22;8(January):1–13. ; Newby GA, Yen JS, Woodard KJ, Mayuranathan T, Lazzarotto CR, Li Y, et al. Base editing of haematopoietic stem cells rescues sickle cell disease in mice. Nature. 2021 Jul 8;595(7866):295–302; Dimitrievska M, Bansal D, Vitale M, Strouboulis J, Miccio A, Nicolaides KH, El Hoss S, Shangaris P, Jacków-Malinowska J. Revolutionising healing: Gene Editing's breakthrough against sickle cell disease. Blood Rev. 2024 May;65:101185. doi: 10.1016/j.blre.2024.101185. Epub 2024 Mar 7. PMID: 38493007; Shangaris P, Loukogeorgakis SP, Subramaniam S, Flouri C, Jackson LH, Wang W, Blundell MP, Liu S, Eaton S, Bakhamis N, Ramachandra DL, Maghsoudlou P, Urbani L, Waddington SN, Eddaoudi A, Archer J, Antoniou MN, Stuckey DJ, Schmidt M, Thrasher AJ, Ryan TM, De Coppi P, David AL. In Utero Gene Therapy (IUGT) Using GLOBE Lentiviral Vector Phenotypically Corrects the Heterozygous Humanised Mouse Model and Its Progress Can Be Monitored Using MRI Techniques. Sci Rep. 2019 Aug 12;9(1):11592. doi: 10.1038/s41598-019-48078-4. Erratum in: Sci Rep. 2019 Dec 24;9(1):20214. doi: 10.1038/s41598-019-55754-y. PMID: 31406195; PMCID: PMC6690943.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -87-

Supervisor(s): Jernej Ule	**E-mail (lead supervisor):** jernej.ule@kcl.ac.uk

Project title: The roles of nuclear speckles in neuronal homeostasis and neurodegeneration

Research area (keywords): molecular neurobiology, neurodegeneration, protein-RNA complexes

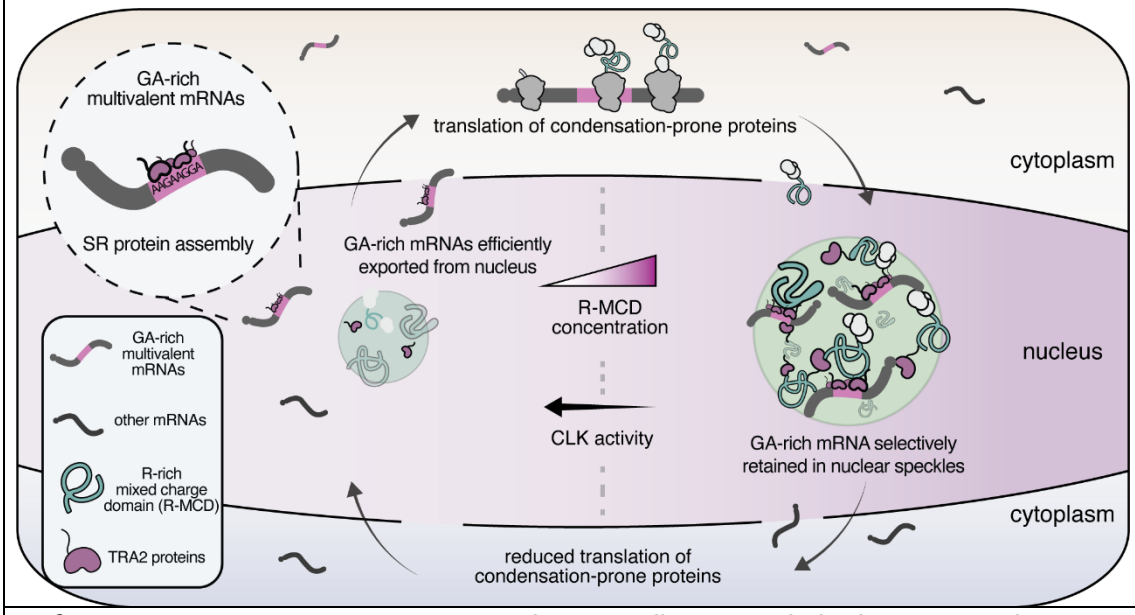
Research skills (keywords): culture and differentiation of human induced pluripotent stem cells, protein and RNA imaging (single-molecule FISH), protein-RNA interaction studies with transcriptomics, computational data analysis

Faculty/School/Department: Department of Basic and Clinical Neuroscience, IoPPN

Location: Maurice Wohl Clinical Neuroscience Institute

Project description: The student will join a team of researchers in the Laboratory of RNA networks (https://ukdri.ac.uk/team/jernej-ule) to work on the role of nuclear speckles in neurons in the context of genetic mutations that cause amyotrophic lateral sclerosis (ALS). The project will build on our recent discovery of 'interstasis' (described in the reference below, and schematised in the figure), a mechanism where the protein-RNA interactions within speckles are altered in such a way that they control the dosage of condensation-prone nuclear proteins. This promotes the homeostasis of such proteins, and this project will ask how this mechanism may protect from the toxicity of ALS-causing mutations.

The student will contribute to the studies of protein-RNA complexes by using the Ngn2-differentiated iNeurons that contain ALS-causing mutations, studying proteins and RNAs in nuclear speckles and their roles in the ALS-related molecular pathogenesis with the use of imaging (including single-molecular FISH for RNA imaging). Further investigations will be based on molecular reporter assays and transcriptomic studies of protein-RNA interactions.



References: R Faraway, NC Heaven, H Digby, OG Wilkins, AM Chakrabarti, IA Iosub, Knez L, Ameres SL, Plaschka C, Ule J (2023) Mutual homeostasis of charged proteins bioRxiv, 2023.08. 21.554177

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -88-

Supervisor(s): Dr Maxim Freydin & Prof Frances Williams	**E-mail (lead supervisor):** maxim.freydin@kcl.ac.uk

Project title: Body proportions as a risk factor for low back pain

Research area (keywords): human and medical genetics, molecular epidemiology, pain

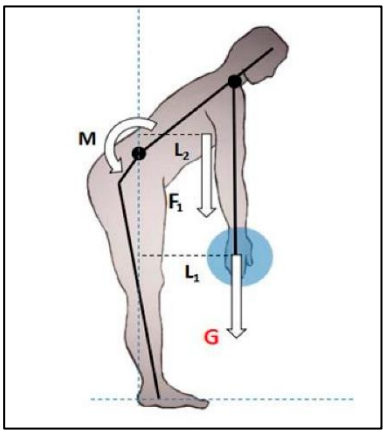
Research skills (keywords): genome-wide association study, genetic correlations, Mendelian randomization, bioinformatics, statistics

Faculty/School/Department: Life Sciences and Medicine / Life Course and Population Sciences / Twin Research and Genetic Epidemiology

Location: St Thomas' Hospital Campus

Low back pain (LBP) is a common debilitating condition and the leading cause of disability worldwide. Both genetic and environmental factors as well as their interaction have been shown to play a role in the risk of LBP and the disorder becoming chronic. Notably, LBP disproportionally affects more females than males, but the reasons for this sex-disparity are not known.

This project aims to investigate **our hypothesis** that the higher risk of LBP among females is based on the different body proportions between the sexes; namely, the relatively longer torso than legs in females, compared to males. From the biomechanical point of view, in case of the longer torso, low back muscles would experience stronger tension upon bending and heavy lifting; thus increasing the risk of injury and leading to LBP.



Specifically, the project will include the **sex-stratified analysis** of associations between chronic LBP on one hand and height, sitting height, torso length and other measurements of this kind on the other hand. Additional exposure factors will be considered, such as job including heavy lifting. **GWAS** followed by the analysis of **genetic correlations** between LBP and anthropometric measurements will be carried out. **Mendelian randomization** will be used to add value to the hypothesis about the causal impact of body proportions on the risk of chronic LBP. **Polygenic risk scores** for body proportions will be developed and tested against the risk of chronic LBP in the sex-stratified fashion.

A prospective student will develop and advance **a wide range of analytical skills** such as programming in R and bash, statistical analysis of large-scale population data, GWAS and genetic correlations, polygenic risk scores, and Mendelian randomization. The project will be hosted within the Department of Twin Research and Genetics Epidemiology. It is a **computational project** based on the use of publicly available datasets such as UK Biobank, FinnGen, and TwinsUK; there is with no wet experimental work involved.

References: Freidin et al. Insight into the genetic architecture of back pain and its risk factors from a study of 509,000 individuals. 10.1097/j.pain.0000000000001514; Freidin et al. Sex- and age-specific genetic analysis of chronic back pain. 10.1097/j.pain.0000000000002100.

MSc/MRes Biomedical & Molecular Sciences Research

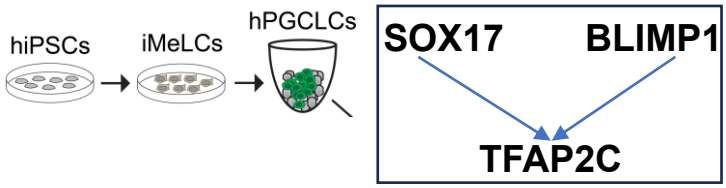
MSci Molecular Genetics

Research Project -89-

Supervisor(s): **Dr. Mahesh Sangrithi**	**E-mail (lead supervisor):** mahesh.sangrithi@kcl.ac.uk
Project title: Refinements in hIPSCs reprogramming toward primordial germ cell fate	
Research area (keywords): hiPSC, stem cells, germ cells, reprogramming,	
Research skills (keywords): nucleic acid manipulation, gene expression, PCR, RTPCR, qPCR, immunofluorescence	
Faculty/School/Department: FoLSM / BMBS / CGTRM	
Location: 28th floor Guy's Tower	

Project description:

The correct specification and development of the human germline is crucial to ensure normal fertility and embryonic development. Despite this important role, early molecular mechanisms regulating human primordial germ cell (hPGC) development remain only partly understood. And because hPGCs arise very early-on during human development at ~Wk3, it remains very challenging to perform such studies on *in vivo* samples. Therefore *in vitro* derived human PGC-like cells (hPGCLCs) serve as useful tool for further work. Previous studies have identified human germ cell specific transcription factors (TFs), that can induce hPGCLCs via differentiating through an intermediate step of incipient mesoderm-like cells (iMELCs). And early studies have shown that these TFs may be employed to reprogramme human pluripotent stem cells into hPGCLCs. Achieving correct expression levels for these TFs also appears to be a key driver of success. A key question that remains unanswered is if trans-differentiation techniques may be optimized further, with an aim to circumvent traditional gene delivery methodology using viral transduction or plasmid transfection. The success of such techniques will significantly improve the generation of patient-specific hPGCLCs to better model infertility related disorders. This project will involve: i) reprogramming in-house available h-iPS cell line with fluorescent reporters toward PGCLC fate, ii) the candidate will further use immunofluorescence microscopy using a set of validated antibodies to test for the expression of additional PGC markers (e.g. BLIMP1, UTF1, TFAP2C, PRDM14) reprogramming timing and efficiency. Contrast with expression of endoderm markers (e.g. FOXA2). RNA will be collected over a time-course of ~ 5-7days, iii. Success of reprogramming will be assessed based on the expression of stage-specific fluorescent reporters, iv. FACS based purification may be performed followed by RT-qPCR will be performed to test for transcriptional, v. Reprogramming dynamics will be further compared to less efficient methods using cytokines alone.



References:

1. doi:10.1038/nature22812 ; 2. doi: 10.1016/j.mce.2023.111949

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -90-

Supervisor(s): Mario Falchi	**E-mail (lead supervisor):** mario.falchi@kcl.ac.uk

Project title: Identification of early instigators of inflammaging in the TwinsUK cohort

Research area (keywords): computational medicine; immunology; inflammation

Research skills (keywords): computational and statistical skills

Faculty/School/Department: SLCPS, Department of Twin Research and Genetic Epidemiology

Location: St Thomas' Hospital

Project description:

The relationship between circulating immune cells and key proteins involved in inflammatory diseases and conditions has been extensively studied in immunology, offering crucial insights into the body's response to infection, injury, and chronic conditions. However, many studies have focused on diseased subjects, often at advanced stage of the disease, and our understanding of these interactions in the general aging population remains limited. Gaining insights into this area could help identifying early triggers of inflammaging, the chronic low-grade inflammation associated with aging that is a major contributor to age-related diseases.

The TwinsUK cohort is a well-characterised study of thousands of adult twins with extensive clinical, molecular and environmental data. In 500 twins we have characterised through flow cytometry a high-resolution characterisation of the major lymphocyte subsets, capturing nearly 80,000 cell types (Figure 1), and 74 proteins related to immune response, inflammation, and immune system regulation. This project will systematically investigate the network of interactions between immune cells and inflammatory proteins to identify key players driving age-related changes in inflammation and immune response.

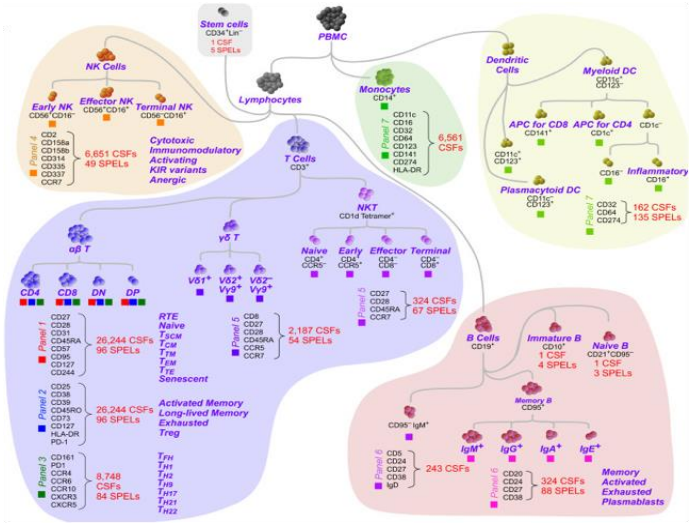


Figure 1. Schematic representation of the characterised leukocyte populations. From Roederer et al 2015

Final aim is to identify early instigator of inflammation, which may provide targets for interventions to slow or mitigate inflammaging. This in-silico project will involve a range of analytical methods, providing essential skills for a future career in computational medicine.

References: Roederer M, Quaye L, Mangino M, Beddall MH, Mahnke Y, Chattopadhyay P, Tosi I, Napolitano L, Terranova Barberio M, Menni C, Villanova F, Di Meglio P, Spector TD, Nestle FO. The genetic architecture of the human immune system: a bioresource for autoimmunity and disease pathogenesis. Cell. (2015) Apr 161:387-403

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -91-

Supervisor(s): Connor Sproston, Ryan Cheng and Darren Williams.	**E-mail (lead supervisor):** darren.williams@kcl.ac.uk

Project title: Revealing the molecular control of programmed cell death in the nervous system of the firebrat *Thermobia domestica*

Research area (keywords): Stem cells, neuronal lineages, Neurodevelopment, programmed cell death, evolution.

Research skills (keywords): Molecular biology, genomics, HCR, CRIPSR/Cas9 and confocal imaging.

Faculty/School/Department: IOPPN/Neuroscience/Centre for Developmental Neurobiology

Location: Centre for Developmental Neurobiology, 4th Floor, New Hunt's House

Project description: The Firebrat *Thermobia domestica* is a member of the Zygentoma and represents one of the most ancestral lineages of insects [1]. During the Devonian, insects diverged from their zygentoman ancestors, evolved wings and metamorphosis. This ability to fly lead to an explosive speciation resulting in insects being the most species-rich group on the planet. Our recent work on flies suggests that programmed cell death plays a key role in development in the development of the nervous system and the evolution of novel circuit motifs [2,3]. This project focuses on exploring developmental death in the 'living fossil' *Thermobia*. We will use state-of-the-art approaches, including CRIPSR/Cas9 to knock out cell death genes, perform RNAseq to find key death regulators and then visualise them with Hybridization Chain Reaction HCR *in situ* approaches using confocal microscopy. We have all the tools in hand make some very important and publishable discoveries.

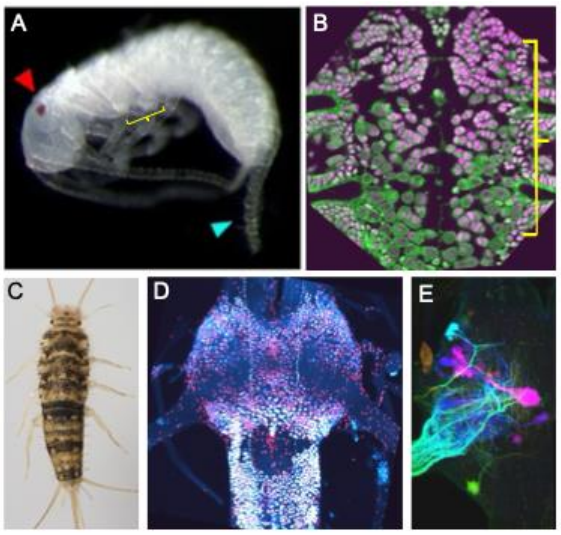


Figure 1. The development and neuroanatomy of *Thermobia domestica*. A. Day 8 *Thermobia* embryo. Yellow bracket shows location of developing thoracic nervous system. **B.** Staining revealing developing ventral nervous system with neuroblasts and neuronal lineages labelled in a day 8 embryo. Yellow bracket shows location in relation to embryo. **C.** *Thermobia* adult. Primitive wingless, ametabolous insect. **D.** The third thoracic ganglia of adult showing staining against specific cell markers. **E.** Dye backfilling of leg nerve revealing processes of sensory neurons and motor neurons in the CNS (colour indicates position within the dorsoventral axis).

References:

1. https://doi.org/10.1126/science.1257570
2. https://doi.org/10.7554/eLife.59566
3. https://doi.org/10.1101/2024.02.11.579841
4. https://doi.org/10.1016/j.asd.2018.06.003

MSc/MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -92-

Supervisor(s): Dr Peter Hill (PI), Dr Andrea Majstorovic	**E-mail (lead supervisor):** peter.w.hill@kcl.ac.uk

Project title: CRISPR screen to identify host factors enabling *Salmonella* survival during antibiotic treatment

Research area (keywords): host-pathogen interactions; antibiotics; CRISPR screens

Research skills (keywords): infection biology; cellular/molecular biology; CRISPR, bioinformatics

Faculty/School/Department: FoLSM / SIMS / Department Infectious Diseases

Location: Guy's Hospital, Borough Wing, 3rd floor

Project description:

Background: Non-typhoidal *Salmonella enterica* infects ~93 million people annually. While non-typhoidal *Salmonella* infections are normally restricted to the gastrointestinal tract, in immunocompromised patients they can cause an invasive (i.e. bloodstream) disease that is associated with a 20% mortality rate. Although antibiotic treatment relieves acute symptoms of invasive *Salmonella* disease, infections often relapse. Relapse of infection is both dangerous for an individual patient and is associated with the rise of antibiotic resistance, making it a major issue for healthcare systems. Relapse of infection is due to small reservoirs of bacteria called persisters that are found in a patient during antibiotic treatment. Persisters are genetically antibiotic-sensitive bacteria that transiently acquire low drug sensitivity after they are phagocytosed by patient macrophages during infection. To prevent relapse of invasive *Salmonella* disease, it is essential to develop strategies to kill persisters. Our group and others have shown that host macrophage phenotype affects *Salmonella* persister survival, although the exact relationship between these remains unresolved. Identifying which macrophage factors affect persister survival will help identification of novel therapeutic targets to prevent *Salmonella* infection relapse. In turn, this will help stop the rise of antibiotic resistance.

Aim: The aim of this project is to perform a CRISPR interference (CRISPRi) screen to identify host factors affecting *Salmonella* survival and proliferation in human macrophages (specifically THP1-derived macrophages). The relevant CRISPR-dCas9-ZIM3 THP1 cell line has already been constructed and validated; therefore, the student will be able to focus immediately on optimisation of infection parameters to determine robust and reproducible infection conditions for detecting relevant macrophage sub-populations, including: macrophages containing killed *Salmonella*; macrophages containing live persisters; and macrophages containing proliferating bacteria. These assays will be performed using validated *Salmonella* fluorescent reporter strains and validated protocols for macrophage isolation by fluorescence-activated cell sorting (FACS). Once optimised, the student will use the CRISPR-dCas9-ZIM3 THP1 cell line to perform a CRISPRi screen to identify genetic knockdowns enriched in each *Salmonella*-infected macrophage sub-population. The student will then validate hits. Finally, the student will characterise validated hits using next-generation sequencing, immunolabelling and classic biochemical/molecular biology/cell biology approaches.

Methods: The project will provide an opportunity for the student to learn an array of wet lab and bioinformatic methods. Students will be trained in techniques such as: infection of human macrophage cell lines; antibiotic treatment assays; FACS; CRISPRi and associated bioinformatic methods; RNA-seq; and biochemical, molecular and cell biology-based approaches.

References: Stapels et al. (Science 2018) *Salmonella* persisters undermine host immune defenses during antibiotic treatment. DOI: https://doi.org/10.1126/science.aat7148; Hill et al. (Cell Host Microbe 2021) The vulnerable versatility of Salmonella antibiotic persisters during infection. DOI: https://doi.org/10.1016/j.chom.2021.10.002; Hill and Helaine (Persister Cells and Infectious Disease 2019) Antibiotic Persisters and Relapsing *Salmonella enterica* Infections. DOI: https://doi.org/10.1007/978-3-030-25241-0_2.

Projects 93-110

are suitable for:

MSc students

MRes students

MSci Biochemistry students

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project - 93 -

Supervisor(s): Dr Richard Parsons, Tong Kang, Prof. Stephen Stürzenbaum	**E-mail (lead supervisor):** richard.parsons@kcl.ac.uk

Department: Faculty of Life Sciences & Medicine, King's College London

Location: Franklin Wilkins Buildings, Waterloo Campus, Labs 5.25, 5.29 and 3.123

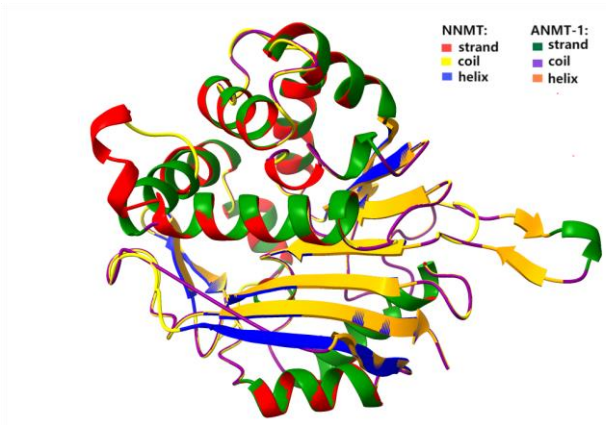
Project title: From worms to man: investigating how mammalian proteins have evolved from *C.elegans*.

Research area: molecular genetics, molecular evolution, cellular homeostasis, *in vivo* models of human diseases

Keywords: *C. elegans*, molecular evolution, RNAseq, GSEA, *in vivo* disease models

Project description:

In humans, nicotinamide homeostasis is maintained by NNMT. This enzyme plays a central role in cellular homeostasis, and its dysregulation is key to the progression of many diseases including cancer, neurodegenerative disease, obesity and diabetes[1]. We have developed and patented several inhibitors of NNMT for treatment of human diseases[2]. Using molecular genetics approach we have dated the first appearance of the NNMT gene to fish. Consequently, we have identified its evolutionary path through the land to sea transition and the effects this evolution has had upon the function and regulation of the protein.



We have compared the 3D structures of human NNMT and C. elegans ANMT-1. Despite their lack of sequence homology, both proteins show areas of structural identity as well as sharing the same catalytic residue, Y20(NNMT)/Y35(ANMT-1).

Our aim is to go even further back in evolutionary time to identify the ancestor of the NNMT gene. It is reported that *C. elegans* expresses a protein called ANMT-1, which may be the prime candidate. Activity assays on recombinant protein show that although nicotinamide is a very poor substrate for ANMT-1, it is still active, suggesting that this may be the ancestral form we are seeking. This project will use RNAseq, gene set enrichment analysis, target gene specific transcriptomic analysis by qPCR and functional assays using *in vitro* molecular cloning to determine whether *C. elegans* regulate nicotinamide biochemistry using ANMT-1. The training provided in these state-of-the-art techniques, will create transferable skills that can be applied to most basic biomedical and health research. The knowledge obtained will be essential in generating *in vivo* models of human disease which can be used to high-throughput screen NNMT-targeting therapies. Furthermore, the project will improve our understanding of how the evolution of nicotinamide metabolism in the cell has contributed to cellular homeostasis, increased organismal complexity, and human disease.

References: [1] Parsons RB, Facey PD (2021) Biomolecules 11: 1418 **[2]** Gao Y et al. (2021) J Med Chem 64: 12938-12963

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -94-

Supervisor(s): Anna Zoccarato, Ajay M. Shah	**E-mail (lead supervisor):** anna.zoccarato@kcl.ac.uk

Project title: Metabolic profiling of human induced pluripotent stem cell derived cardiac fibroblast (hiPSC-CF) during pathological activation.

Research area (keywords): Heart failure, cardiac fibrosis, metabolism, hiPSC-CF

Research skills (keywords): Metabolomics, LC-MS, Tissue culture, Bioenergetics

Faculty/School/Department: FoLSM, School of Cardiovascular and Metabolic Medicine & Sciences

Location: BHF Centre of Research Excellence, James Black Centre, Denmark Hill campus

Project description:

Background: Cardiac fibrosis is a common feature in various heart diseases, including both ischemic and non-ischemic heart failure, genetic cardiomyopathies, diabetes, and aging. In response to stress or injury, cardiac fibroblasts activate and differentiate into myofibroblasts, leading to increased collagen and extracellular matrix (ECM) deposition, which contributes to cardiac fibrosis. While initially beneficial for cardiac function and preventing heart rupture, excessive fibrosis causes ECM expansion, myocardial stiffening, reduced nutrient and oxygen delivery, and increased cardiomyocyte death, ultimately resulting in pathological remodelling and heart failure. Understanding the molecular pathways that control cardiac fibroblast activation is crucial for developing heart failure treatments. Although heart metabolism changes are known to be critical in cardiac remodelling and heart failure, little is known about metabolic reprogramming specifically in cardiac fibroblasts during fibrosis (1). This gap in knowledge stems from the limited availability of primary human cardiac fibroblasts. Recently, protocols have been developed to generate human iPSC-derived cardiac fibroblasts (hiPSC-CFs), closely resembling primary CFs (2). We have now established lab protocols to differentiate human iPSCs into cardiac fibroblasts capable of activation and differentiation into myofibroblasts.

Aim: The goal is to study metabolic reprogramming in hiPSC-CFs during stress-induced myofibroblast activation using state-of-the-art metabolomics and [U-^{13}C]glucose and [U-^{13}C]glutamine flux analysis (3). The initial focus will be on developing optimal protocols to assess ^{13}C-metabolite enrichment in hiPSC-CFs. Next, hiPSC-CFs will be stimulated to induce fibroblasts activation, and metabolomic and flux analysis will be performed to study changes in glucose and glutamine metabolism. In addition to metabolomic analysis, bioenergetics, metabolic enzyme expression levels, and other functional readouts including cell contractility will be assessed.

Methods: hiPSC culture and differentiation into cardiac fibroblast (hiPSC-CFs), metabolomics, flow cytometry, immunofluorescence, extracellular flux analysis (Seahorse), qPCR and data analysis. The student will be based within Prof. Ajay M. Shah's team, a highly multi-disciplinary group that studies cardiac remodelling and heart failure with multiple approaches across the spectrum from molecular to in vivo.

References:

1. Gibb AA, et al. "Myofibroblasts and Fibrosis: Mitochondrial and Metabolic Control of Cellular Differentiation." *Circ Res*. 2020;127(3):427-447.
2. Zhang, J. et al. "Functional cardiac fibroblasts derived from human pluripotent stem cells via second heart field progenitors." Nat Commun 10, 2238 (2019).
3. Zoccarato A. et al. "NRF2 activation in the heart induces glucose metabolic reprogramming and mediates cardioprotection via upregulation of the pentose phosphate pathway" bioRxiv 2023.05.12.540342.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -95-

Supervisor(s): Dr Kourosh H. Ebrahimi	E-mail (lead supervisor): kourosh.ebrahimi@kcl.ac.uk

Project title: Discovery of novel broad-spectrum antiviral natural products

Research area (keywords): Drug discovery and enzymology

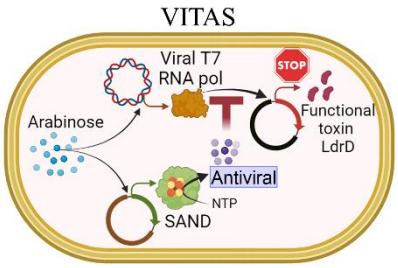
Research skills (keywords): Molecular biology, biochemical techniques, mass spectrometry, NMR spectroscopy

Faculty/School/Department: Faculty of Life Sciences and Medicine, Institute of Pharmaceutical Science

Location: FWB Building, Waterloo Campus

Project description:

Viruses are a global challenge causing a huge socioeconomic burden. Because of the error-prone activity of their replication machinery, they continuously evolve, leading to the emergence and re-emergence of new strains of viruses with higher mortality and infectivity rates and the ability to invade vaccines and many therapeutics. Therefore, there is a growing need to discover and develop new broad-spectrum antiviral drugs to counter future viral pandemics. In this regard, nucleotide/nucleoside analogues are among the most prominent candidates because they target the error-protein activity of viral replication machinery and block viral reproduction. Consequently, they help counter the social and economic costs of emerging viruses like HIV-1, Ebola, and SARS-CoV-2. They are one of the largest groups of antiviral therapeutics (>25 FDA-approved drugs).[1] Some of these drugs are on the World Health Organization's List of Essential Medicines. Hence, the enzymatic production of these drugs is vital for building a net-zero emission economy.



We have recently discovered a new class of enzymes capable of producing antiviral lead nucleotide analogues[2,3]. The enzyme has many uncharacterised homologues in all domains of life, and these enzymes are predicted to produce new antiviral lead natural products. To screen the activity of these enzymes and identify new ones, two previous M.Sc. project students developed an in vivo screening assay named VITAS[4] and combined this assay with AI-based protein prediction and molecular dynamics (MD) calculations in collaboration with Prof Maria Andrea Mroginski (TU Berlin). Building on this work, in this project, you will combine VITAS with computational methods to identify new enzymes producing antiviral nucleotide analogues. Their activity and the antiviral nucleotide analogue produced will be characterised using liquid chromatography-mass spectrometry (LC-MS). One selected enzyme will be overexpressed, purified, and characterised using molecular biology methods, protein expression and purification techniques, UV-visible and electron paramagnetic resonance (EPR) spectroscopy, and LC-MS. You will have the opportunity to visit the laboratory of one of our collaborators in Germany or the Netherlands for one month to learn and develop new skills. It is expected that you publish your work as a research article.

References:

[1] L. P. Jordheim, D. Durantel, F. Zoulim, C. Dumontet, *Nat. Rev. Drug Discov.* **2013**, *12*, 447–464.

[2] K. H. Ebrahimi, et al., *FEBS Lett.* **2017**, *591*, 2394–2405.

[3] K. H. Ebrahimi, J. Rowbotham, J. McCullagh, W. S. James, *ChemBioChem* **2020**, *21*, 1605–1612.

[4] Fahd Alharbi, A., et al., VITAS, Chem Comm (2023) 59, 5419-5422.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -96-

Supervisor(s): Prof Chris Miller; Dr Kerry Blair, Dr Raquel Martinez, Dr Philippe Gosset	**E-mail (lead supervisor):** chris.miller@kcl.ac.uk
Project title: Endoplasmic reticulum (ER)-mitochondria signaling as a novel therapeutic target for dementia	
Research area (keywords): Alzheimer's disease; Fronto-temporal dementia; amyotrophic lateral sclerosis; Novel therapeutic target; drug screen	
Research skills (keywords): Molecular biology; neural cell culture and manipulation; advanced microscopy	
Faculty/School/Department: FoLSM/IoPPN/Basic and Clinical Neuroscience	
Location: Denmark Hill; Maurice Wohl Clinical Neuroscience Institute	

Project description:

Alzheimer's disease and related dementias are major diseases that impact dramatically on the lives of patients and carers but also on our economy; the cost of dementia to the UK will be approximately £42 billion in 2024. There are no cures for dementia and new targets for therapeutic intervention are needed. Dementia is characterised by damage to a wide number of neural functions; these include damage to synaptic function, axonal transport and inflammatory responses. It is not clear how such disparate functions are collectively perturbed in dementia and this also raises issues with formulating novel drug targets; which damaged function should be prioritised for therapeutic intervention? Recently, interest has focussed on signaling between the ER and mitochondria since this regulates many of the functions that are damaged in dementia. ER-mitochondria signaling involves close physical contacts between the 2 organelles that are mediated by "tethering" proteins that act to recruit regions of ER to the mitochondrial surface. We have identified a key tether; it involves an interaction between the ER protein VAPB and the mitochondrial protein PTPIP51. We have gone on to show that the VAPB-PTPIP51 tethers are broken in Alzheimer's and related dementias. Thus, disruption of ER-mitochondria signaling via breaking of the VAPB-PTPIP51 tethers may underlie the damage to many other physiological functions in seen in dementia. As such, correcting disrupted ER-mitochondria signaling may be broadly therapeutic. We have recently provided evidence to support this notion. Our findings that VAPB and PTPIP51 are ER-mitochondria tethering proteins that are damaged in dementia identifies a defined new molecular target for drug intervention. This has enabled us to identify novel small molecule drugs that modulate VAPB-PTPIP51 binding. This project is to characterise some of these molecules further. The work will involve determining how such molecules affect functions regulated by ER-mitochondria signaling in dementia. Our work is supported by major grants from the Medical Research Council and Alzheimer's Research UK.

References:

Paillusson, S., et al 2016. Trends Neurosci. 39 146-157. PMID: 26899735

Martín-Guerrero, S.M., et al. 2022. Front Cell Dev Biol. 10 915931 PMID:35693938

Markovinovic, A., et al 2024. Acta Neuropath. Comm. 12, 32. PMID:38395965

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -97-

Supervisor(s): Prof Chris Miller; Dr Kerry Blair, Dr Raquel Martinez, Dr Philippe Gosset	**E-mail (lead supervisor):** chris.miller@kcl.ac.uk
Project title: Structural studies of Endoplasmic reticulum (ER)-mitochondria signaling in dementia	
Research area (keywords): Alzheimer's disease; Fronto-temporal dementia; amyotrophic lateral sclerosis; Novel therapeutic target	
Research skills (keywords): Molecular biology; neural cell culture and manipulation; advanced microscopy; structural biology	
Faculty/School/Department: FoLSM/IoPPN/Basic and Clinical Neuroscience	
Location: Denmark Hill; Maurice Wohl Clinical Neuroscience Institute+Guys' campus	

Project description:

Alzheimer's disease and related dementias are major diseases that impact dramatically on the lives of patients and carers but also on our economy; the cost of dementia to the UK will be approximately £42 billion in 2024. There are no cures for dementia and new targets for therapy are need. Dementia is characterised by damage to a wide number of neural functions; these include damage to synaptic function, axonal transport and inflammatory responses. It is not clear how such disparate functions are collectively perturbed in dementia and this also raises issues with formulating novel drug targets; which damaged function should be prioritised for therapy? We now know that many functions damaged in dementia are regulated by signaling between the ER and mitochondria; ER-mitochondria signaling is thus a highly topical area of research since it impacts on many cellular functions. ER-mitochondria signaling involves close physical contacts between the 2 organelles that are mediated by "tethering" proteins that act to recruit regions of ER to the mitochondrial surface. We identified a key tether; it involves an interaction between the ER protein VAPB and the mitochondrial protein PTPIP51. We have gone on to show that the VAPB-PTPIP51 tethers are broken in Alzheimer's and related dementias and that this involves activation of the kinase GSK3β. GSK3β is strongly implicated in dementia. Since phosphorylation is a mechanism for regulating protein-protein interactions, one notion is that activation of GSK3β in dementia leads to phosphorylation of either VAPB and/or PTPIP51 to inhibit their binding and so disrupt ER-mitochondria signaling. This project aims to gain insight into the structural features that regulate VAPB-PTPIP51 and how phosphorylation impacts on this. The project will involve the identification and experimental manipulation of phosphorylation sites and determining how this affects ER-mitochondria signaling in dementia. Our work is supported by major grants from the Medical Research Council and Alzheimer's Research UK.

References:

Paillusson, S., et al 2016. Trends Neurosci. 39 146-157. PMID: 26899735

Martín-Guerrero, S.M., et al. 2022. Front Cell Dev Biol. 10 915931 PMID:35693938

Markovinovic, A., et al 2024. Acta Neuropath. Comm. 12, 32. PMID:38395965

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -98-

Supervisor(s): Katarzyna Kmiotek-Wasylewska, Anna Zoccarato	**E-mail (lead supervisor):** anna.zoccarato@kcl.ac.uk

Project title: The role of extracellular vesicles (EVs) derived from cardiac fibroblasts (iPS-CFs) in development of cardiac hypertrophy *in vitro*.

Research area (keywords): cardiac hypertrophy, EVs, hiPS-CMs, hiPS-CFs, metabolism

Research skills (keywords): tissue culture, cell differentiation, EVs isolation, flow cytometry

Faculty/School/Department: SCMMS

Location: Denmark Hill Campus

Project description:

Background: Prolonged exposure of the heart to pathological stress or injury induces cardiac remodelling, a process that can culminate in heart failure (HF), a leading global cause of mortality. Several studies have elucidated the important role of metabolic alterations in cardiac myocytes, and showed how these changes drive the progression of cardiac hypertrophy. Extracellular vesicles (EVs) are small membrane-bound particles released by all cell types and they play a crucial role in intercellular communication. EVs encapsulate a variety of bioactive molecules, including RNA (mRNA, lncRNA, miRNA), proteins, and lipids. This bioactive cargo can be transferred to recipient cells, influencing their phenotype and functional activity. However, the involvement of EVs in the development of cardiomyocyte hypertrophy remains largely unexplored. Emerging evidence suggests that EVs may facilitate crosstalk between cardiomyocytes and cardiac fibroblasts during hypertrophy [1], with the miRNA (miR-21, miR-27a) content of EVs derived from cardiac fibroblasts potentially promoting hypertrophic phenotypes in recipient cardiomyocytes via impact on expression of genes related to hypertrophy such us ACTA1, MYH6, MYH9 and COL1A1 [2]. As for now, however, here is no evidence that EVs from cardiac fibroblasts may promote changes in CMs metabolism.

Aim: To investigate the role of EVs in cardiac hypertrophy. The approach is to isolate EVs from hiPS-CFs under both control and stress conditions (we have established protocols to activate hiPSC-CFs to myofibroblast with 10nM endothelin (ET-1) which is a known pro-hypertrophic stimuli) using sequential centrifugation with ultracentrifugation step. We will characterize vesicles according to ISEV guidelines [3] and investigate the RNA and protein content of these vesicles, with a focus on genes associated with hypertrophy (e.g., ANP, BNP) and glucose metabolism (e.g., PFKP, PGAM2, PKM2). Finally, we will assess the development of hypertrophic phenotypes in iPS-CM following EV stimulation, utilizing techniques routinely employed in our laboratory such as flow cytometry, mRNA and protein analysis.

Methods: hiPSC culture and differentiation into hiPSC-CMs and hiPS-CFs, EVs isolation and characterisation, metabolomics, flow cytometry, western blotting, qPCR and data analysis. The student will be based within Prof. Ajay M. Shah's team, a highly multi-disciplinary group that studies cardiac remodelling and HF with multiple approaches across the spectrum from molecular to *in vivo*.

References:

1. Mallaredy V. et al. "Tipifarnib Reduces Extracellular Vesicles and Protects From Heart Failure" Circulation Research vol. 135,2 (2024): 280-297
2. Kamo T. et al. "Cardiac Nonmyocytes in the Hub of Cardiac Hypertrophy" Circulation Research vol. 117,1 (2015): 89-98
3. Welsh JA. Et al. "Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches" J Extracell Vesicles vol. 12,2 (2024): e12404

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -99-

Supervisor(s): Dr Joseph Burgoyne	**E-mail (lead supervisor):** joseph.burgoyne@kcl.ac.uk

Project title: Promoting cardiac regeneration by targeting the redox sensor in NEK1

Research area (keywords): Cell signalling, redox biology, cardiac regeneration

Research skills (keywords): Molecular biology, cell biology

Faculty/School/Department: School of Cardiovascular and Metabolic Medicine & Sciences

Location: The Rayne Institute, St Thomas' Hospital, Westminster Bridge Road

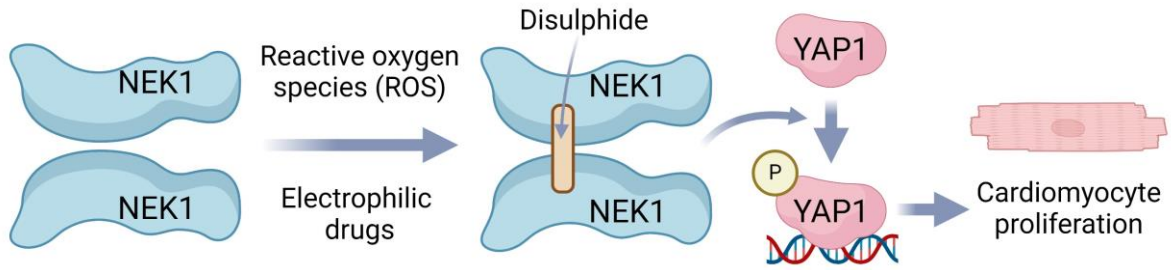
Project description:

Redox sensors have evolved as an important mechanism for transducing changes within the cellular redox environment into a signalling response through alterations in protein function. These sensors are located within key enzymes that include kinases such as PKA and PKG, with their oxidation regulating angiogenesis and blood pressure respectively[1,2]. Given their functional importance and amenability to chemical modification redox sensors provide key sites for therapy[3].

Our group has developed a novel structural bioinformatic screen to identify new redox sensors that can be directly targeted for therapy, including one located within NIMA-related kinase 1 (NEK1). This redox sensor within NEK1 has important translational and therapeutic potential, as this kinase has recently been found to interact and promote the stabilisation and transcriptional output of the Hippo signalling effector YAP1[4]. The activation of YAP1 promotes cardiomyocyte proliferation and cardiac regeneration[5]. Therefore, by targeting the redox sensor in NEK1 this may provide a novel therapy to promote YAP1 activation and cardiac regeneration following injury.

The aim of this project is to characterise the novel redox sensor within NEK1. Specific objectives include,

- Assessing the impact of knocking out NEK1 (using CRISPR-Cas9) on YAP1 activity
- Expressing mutant 'redox-dead' NEK1 to assess the impact of the redox-sensor on YAP1 activity
- Assessing if the redox-sensor in NEK1 can regulate cardiomyocyte proliferation
- Using RNAseq and next generation sequencing to identify other pathways regulated by NEK1



References:

1. Burgoyne JR, et al. Cysteine redox sensor in PKGIa enables oxidant-induced activation. Science 2007.
2. Burgoyne JR, et al. Deficient angiogenesis in redox-dead Cys17Ser PKARIα knock-in mice. Nat Commun 2015
3. Prysyazhna O, et al. Blood pressure-lowering by the antioxidant resveratrol is counterintuitively mediated by oxidation of cGMP-dependent protein kinase. Circulation 2019
4. Khalil M, et al. NEK1 phosphorylation of yap promotes its stabilization and transcriptional output. Cancers (Basel) 2020
5. Xin M, et al. Hippo pathway effector Yap promotes cardiac regeneration. Proc Natl Acad Sci U S 2013

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MSci Biochemistry

Research Project -100-

Supervisor(s): Dr Cinzia Imberti, Dr George Firth	**E-mail (lead supervisor):** cinzia.imberti@kcl.ac.uk

Project title: Enhancing immunotherapy efficacy in neuroblastoma using copper depletion

Research area (keywords): paediatric cancers (neuroblastoma), metallomics, immunotherapy, radionuclide imaging

Research skills (keywords): cell culture, cell uptake assays, radio-immunoconjugate preparation, flow cytometry, inductively coupled plasma mass spectrometry (ICP-MS)

Faculty/School/Department: FoLSM, Department of Imaging Chemistry and Biology

Location: 4th floor Lambeth Wing, St Thomas Hospital

Background: Neuroblastoma is the most frequent extra-cranial solid cancer in children with a five-year survival rate lower than 50% in high-risk disease patients.[1] The disialoganglioside GD2 antigen is a key therapeutic target in neuroblastoma and immunotherapies based on the anti-GD2 antibody Dinutuximab-beta are currently standard of care in high-risk patients.

Copper is an essential micronutrient which has been implicated in cancer growth and invasion.[2] Copper depletion using the FDA-approved chelator tetraethylenetetramine (TETA) was found to improve efficacy of the anti-GD2 antibody Dinutuximab-beta in vivo in immunocompetent mouse models of neuroblastoma.[3] Recent studies in neuroblastoma and glioblastoma have also shown that this may be related to increased GD2 expression in cancer cells.[4,5]

Proposed project: We propose to investigate the ability of TETA and other clinically relevant copper chelators (tetrathiomolibdate and D-penicillamine) to reduce copper levels and increase efficacy of the anti-GD2 antibody Dinutuximab-beta in neuroblastoma cell lines with different GD2 expression.

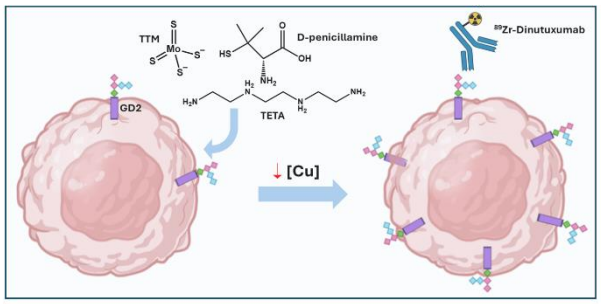

Figure 1. Treatment with copper chelators may enhance uptake of ^{89}Zr-Dinutuxumab by increasing GD2 expression.

We will first measure the effect of copper depletion on basal copper level (using ICP-MS) and cellular accumulation of exogenous copper (using radioactive copper ^{64}Cu). GD2 membrane expression will be measured using flow cytometry and correlated to intracellular copper levels. We will then use standard immunoconjugation and labelling techniques[6] to prepare a radioactive version of Dinutuximab-beta (^{89}Zr-Dinutuxumab) and measure its uptake in these cell lines (including internalised fraction) at different time points after copper depletion. If time allows, and one of the investigated chelators shows promise in increasing ^{89}Zr-Dinutuxumab uptake in vitro, a radionuclide imaging experiment will be performed in animal models of neuroblastoma, comparing tumour uptake of radiolabelled Dinutuximab-beta in mice treated with the copper chelator or saline as a control.

References: 1) B. Qiu and K. K. Matthay, *Nat. Rev. Clin. Oncol.*, 2022 **2)** E. J. Ge et al. Nat Rev. Cancer, 2022. **3)** F. Voli, et al., Cancer Res., 2020 **4)** J. R. Rouaen, et al., Cancer Res. 2024 **5)** T. Shai-Hee, et el., Cancer Res. 2024 **6)** C. Imberti, et al., J. Nucl. Med., 2024.

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MSci Biochemistry

Research Project -101-

Supervisor(s): Maria Jimenez-Sanchez	**E-mail (lead supervisor):** maria.jimenez_sanchez@kcl.ac.uk
Project title: Investigating the link between proteostasis and inflammation in astrocytes	
Research area (keywords): Neurodegeneration, proteostasis, astrocytes, chaperones	
Research skills (keywords): Cell culture, primary glial cultures, western blot, elisa, reporter assays	
Faculty/School/Department: IoPPN/Neuroscience/Basic and Clinical Neuroscience	
Location: Maurice Wohl Neuroscience Institute, Denmark Hill	

Project description:

In neurodegenerative diseases such as Alzheimer's disease (AD), astrocytes become reactive and undergo morphological, functional and molecular changes. Reactive astrocytes contribute to neuron toxicity in neurodegeneration through cell and non cell autonomous effects (Escartin et al., 2021). Single cell transcriptomic studies have highlighted an increase in proteostasis genes in astrocytes in human AD brain, including upregulation of molecular chaperones. This project aims to study how proteostasis is regulated in reactive astrocytes, and the implications of increased chaperone levels in the control of the astrocyte inflammatory response.

Aim 1. Generation of a model of astrocyte reactivity in culture. Treatment of mouse primary astrocytes with a cocktail of pro-inflammatory cytokines (TNFa and IL1a) have been used in our lab to model astrocyte reactivity in culture (Yang et al. 2024). These cultures require serum, which alters the astrocyte transcriptome and prevent from mimicking their physiological response. As a first step in the project, we will first establish and validate a model recently described (Clayton et al., 2024), replacing serum for 4 defined components, which will provide a valuable model to study astrocyte inflammatory response in subsequent aims.

Aim 2. Elucidate changes in the heat shock response in reactive astrocytes. We will next investigate the effect that pro-inflammatory cytokines have on the heat shock response by using a HSE luciferase reporter construct. Using western blotting, we will determine changes in inducible heat shock proteins, particularly focusing on the family of small heat shock proteins (sHSPs) whose expression in glial cells is linked to neurodegenerative diseases. This effect will be complemented with other stimuli that induced astrocyte reactivity such as treatment of astrocytes with media from activated microglia.

Aim 3. Investigate the role of chaperones in preventing the astrocyte inflammatory reaction. HSPB8 belongs to the family of inducible sHSPs and we have generated data suggesting that this chaperone ameliorates the reactive astrocyte phenotype observed upon treatment with TNFa and IL1a. Together with other chaperones of interest identified in Aim 2, we will investigate how HSPB8 can mediate this response, through experiments that include investigating changes in transcriptional response, protein degradation pathways or altered protein secretion. To deep into the effect of HSPB8 on reactive astrocytes, the student will be encouraged to apply to a Matt Wilson scholarship to obtain additional funds to perform proteomics assays to detect the HSPB8 transient interactome.

References:

Yang F… Jimenez-Sanchez M. Reactive astrocytes secrete the chaperone HSPB1 to mediate neuroprotection. Sci Adv. 2024

Clayton et al. A phenotypic screening platform for identifying chemical modulators of astrocyte reactivity. Nat Neurosci. 2024 Apr;27(4):656-665.

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MSci Biochemistry

Research Project -102-

Supervisor(s): Dr Olena Rudyk	**E-mail (lead supervisor):** olena.rudyk@kcl.ac.uk
Project title: Metabolic regulation of redox environment in hypoxic lung	
Research area (keywords): cardiovascular, molecular and cell biology, redox biology, cell signalling, vascular hyper-proliferative disease	
Research skills (keywords): molecular biology, cell biology, metabolomics	
Faculty/School/Department: FoLSM, School of Cardiovascular and Metabolic Medicine & Sciences	
Location: The Rayne Institute, St Thomas' Hospital, Westminster Bridge Road, SE1 7EH	

Project description:

Pulmonary Hypertension (PH) is a devastating vascular disease of high blood pressure in lung arteries and progressive right heart hypertrophy leading to rapid onset heart failure and eventual death. PH pathology is driven by progressive pseudo-malignant inward remodelling and vessel obliteration caused by the expansion of cells in all pulmonary vascular wall layers. Indeed, endothelial, smooth muscle cells and fibroblasts isolated from the lungs of PH patients resemble striking similarities to cancer cells with increased proliferative potential, resistance to apoptosis, dedifferentiated phenotype, cell cycle dysfunction, adapted cellular energetics and metabolism. However, despite cell proliferation being among the most significant features in PH pathology, there is still a lack of detailed understanding of underlying causes or signalling pathways. Therefore, in contrast to systemic hypertension (well-managed with blood-pressure-lowering drugs), there is a highly unmet clinical need for novel true disease-modifying therapies to reverse PH-related remodelling, reopen obliterated vessels and unload the heart to save the lives of PH patients.

ROS are by-products of cellular metabolism, and while historically considered harmful, they are also important mediators of physiological redox signalling. ROS cause reversible posttranslational protein modifications, which can prevent maladaptive disease progression. Our lab investigates ROS-induced modifications of protein cysteine residues and their functional roles in PH. Despite the commonly accepted view and our previous data on increased ROS production in PH, we found that the redox state of functional cysteines in some kinases in pulmonary arteries or lungs of PH patients is more likely to be *reduced* than *oxidised* compared with the healthy donor tissues, suggesting a more intricate and micro-compartmentalised regulation of cysteine oxidation in a disease scenario.

We performed a semi-targeted metabolomics study in lung tissues isolated from mice with hypoxia-induced PH, compared with the normoxic controls, and observed altered branched amino acids, N-acetylglucosamine (GlcNAc) and potentiated TCA cycle in hypoxic lungs correspondingly. The proposed project will validate and build on these findings to explore the metabolic micro-environment in human pulmonary vascular cells in hypoxia. This will further our understanding of the link between hypoxia, metabolism, redox environment and its role in causing PH. Cell culturing, immunoblotting, *in vitro* metabolomic assessment, gene silencing and cell proliferation assays will be performed, among others, as well as NMR or LC/MS assessments of pulmonary vascular cells isolated from healthy donors or PH patients.

References: PMID: 37955182; PMID: 33788196; PMID: 31186362; PMID: 35204311; PMID: 31406951.

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MSci Biochemistry

Research Project -103-

Supervisor(s): Manolis Fanto	**E-mail (lead supervisor):** manolis.fano@kcl.ac.uk

Project title: Molecular characterization of origin and composition of karyoptotic extracellular vesicles

Research area (keywords): Neuroscience, Biochemistry

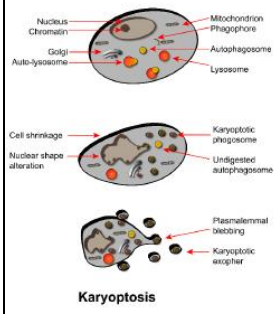
Research skills (keywords): Biochemistry

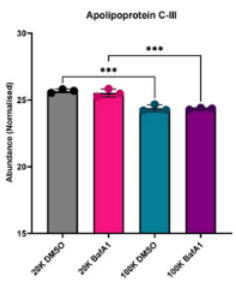
Faculty/School/Department: IoPPN- Basic and Clinical Neuroscience

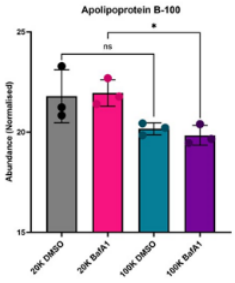
Location: Maurice Wohl Clinical Neuroscience Institute

Project description:

Neurodegenerative diseases like Alzheimer's Disease (AD) or Fronto Temporal Dementia (FTD) are characterized by extensive neuronal cell death, spreading in several areas of the nervous system. Crucially, the type(s) of cell death by which neurons die in dementia has been a long-standing dilemma, which has significantly and negatively impacted the understanding of these pathologies and their potential treatment.







Our recent work has identified and detailed a previously unknown form of regulated cell death, karyoptosis, which may account for the death of a substantial portion of neurons in forms of dementia.In karyoptosis the neurons release large extracellular vesicles (EVs). Proteomic analysis has indicated that these vesicles are rich in nuclear chromatin and lamina components, but also in endoplasmic reticulum (ER) stress markers and apolipoproteins usually found in ER-produced vLDL.

We now wish to validate further the protein content and to detail the nucleic acid components of the karyoptotic EVs, to shed light on their origin and mechanism of formation.

The student will first validate our proteomic data by performing ELISAs for apolipoproteins Apo-B100 and Apo C-III. Thereafter the student will validate the presence of structural nuclear proteins and of proteins related to DNA damage, found enriched in our proteomics analysis, namely Topoisomerase 2B, the RuvBL1 helicase and the DNA damage associated protein MSH-6.

If time allows, one or more additional components, especially related to ER stress will be validated, e.g ERP4, PDIA3 orPDIA4.

In case of an MRes and achievement of a Matt Wilson Scholarship, the student will analyse by high throughput sequencing the DNA content of the karyoptotic EVs and to extract enrichment factors for specific sequences and genomic location, with a view to test the hypothesis that heterochromatin-related DNA are expelled in karyoptotic EVs.

References:

Baron O, *et al.* **Curr Biol.** 2017 Dec 4;27(23):3626-3642.e6. doi: 10.1016/j.cub.2017.10.054. Epub 2017 Nov 22.

Casterton, R, *et al.* Nat Neurosci. Under review (Pre-print ***Research Square*** DOI 10.21203/rs.3.rs-3287063/v1)

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MSci Biochemistry

Research Project -104-

Supervisor(s): James Mason	**E-mail (lead supervisor):** james.mason@kcl.ac.uk

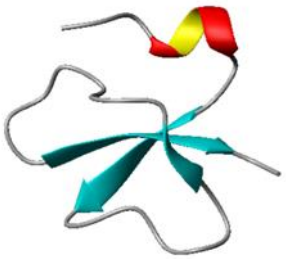
Project title: Are mouse and rat homologues of human beta-defensin 2 functionally distinct?

Research area (keywords): Synthetic chemistry/Microbiology/Biophysics

Research skills (keywords): Solid-phase peptide synthesis/Antibacterial susceptibility testing/CD spectroscopy

Faculty/School/Department: FoLSM/ Cancer & Pharmaceutical Science / IPS

Location: Waterloo - FWB



Project description: hBD-2 is an important protein of the innate immune response providing protection against diverse pathogens, therapeutic administration of which has been proposed as a new approach to treatment in a wide range of indications. However, while hBD-2 is expressed in skin, oral mucosa, lung and several other sites (and can be induced by bacteria and proinflammatory cytokines), expression of the mouse homologue, mBD-4, is restricted to oesophagus, tongue and trachea.[2] Further, while the rat homologue, rBD4, has 52.5% identity and similar physico-chemical properties to the human peptide, including high positive charge and similar hydrophilicity, the mouse homolog has lower identity (48.7%) is less positively charged and is substantially more hydrophobic. Perhaps critically, the human and rat defensins also share an identical sequence "QIGTCGLP" starting at residue #26 whereas the corresponding mouse peptide sequence "QIGNCGHF" has three key differences which will likely impact on the secondary structure of the folded peptide as proline is critical for turns.

The aim of this project is to investigate the extent to which human beta defensin-2 (hBD-2) differs from its supposed mouse and rat homologues to inform the future use of mouse and/or rat models of infection and the development of defensin based therapeutics.

Specific aims of the project include:

1. We will synthesise (solid-phase peptide synthesis) each of the three defensins and use controlled oxidation to obtain the correctly folded β-defensin topology.
2. We will use circular dichroism (CD) spectroscopy to confirm the folding of each of the three defensins and then ask if conformational changes are detected when binding to models of different bacterial and mammalian plasma membranes.
3. We will study the *in vitro* antimicrobial activity of all three peptides in standard microbiological media as well as mammalian cell culture media with human serum to determine whether any or all of the peptides can be genuinely classified as antimicrobial peptides.
4. If time allows we will use the information from 3) to design and execute intervention studies in invertebrate (*Galleria mellonella*) models of infection to understand whether immunomodulatory potential exists alongside any antibacterial capability.

References:

1. Cieślik, M. *et al*. Human β-Defensin 2 and Its Postulated Role in Modulation of the Immune Response. *Cells* 2021 (10) 2991.
2. Peng Jia, H., *et al.* A novel murine β-defensin expressed in tongue, esophagus and trachea *J. Biol. Chem.* 2000 (275) 33314-33320.

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MSci Biochemistry

Research Project -105-

Supervisor(s): Dr Joe Atherton	**E-mail (lead supervisor):** joseph.atherton@kcl.ac.uk

Project title: **Structural bases of microtubule accumulation & hypomyelination in H-ABC leukodystrophies**

Research area (keywords): **Cryo-electron microscopy, structural biology, neuroscience, disease.**

Research skills (keywords): **Cryo-electron microscopy, mutagenesis, cell culture, protein purification, light microscopy.**

Faculty/School/Department: **Randall Centre for Cell & Molecular Biophysics (FoLSM & BMBS).**

Location: **Guy's Campus (New Hunt's House)**

Project background: Oligodendrocytes (OLs) are a glial cell type in the central nervous system (CNS) which, in addition to providing trophic support, wrap neuronal axons in layers of membranous insulating myelin sheathing, allowing fast and efficient neurotransmission. Myelin and/or OL abnormalities or loss leads to neurological diseases including multiple sclerosis and the leukodystrophies. Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) is an inherited childhood leukodystrophy associated with movement abnormalities, spasticity, cerebellar ataxia and sometimes epilepsy. H-ABC is caused by mutations to a β-tubulin isoform, TUBB4A, expressed predominantly in OLs compared to other CNS cell types. Tubulin is the building block of microtubules (MTs) and the mutations investigated so far have been shown to cause a progressive accumulation of MTs in OLs in both a rat model and in human patients, and abnormal hyper-association with endoplasmic reticulum[1,2]. Why these mutations cause MT accumulation and hypomyelination remains unclear, but one hypothesis is that they affect MT structure and dynamics.

Project aims and methods: We have previously shown that via single tubulin isoform expression in insect cells followed by tandem affinity purification, high-resolution cryo-electron microscopy (cryo-EM) studies of MTs built from these isoforms is possible, as well as MT dynamics assays[3]. We have developed user-friendly protocols for processing of cryo-EM data of MTs to high-resolution[4]. The project has the following aims;

1) Use site-directed mutagenesis to make cell expression constructs for TUBB4A tubulin mutations observed in hypo-myelinating leukodystrophies. 2) Express constructs in insect cells and purify single-isoform mutant tubulin dimers using a tandem affinity approach. 3) Perform fluorescence microscopy assays to assess how mutant tubulins influence cellular microtubule dynamics and accumulation. 4) Prepare samples of mutant microtubules for cryo-EM, revealing the structural bases of microtubule hyperaccumulation.

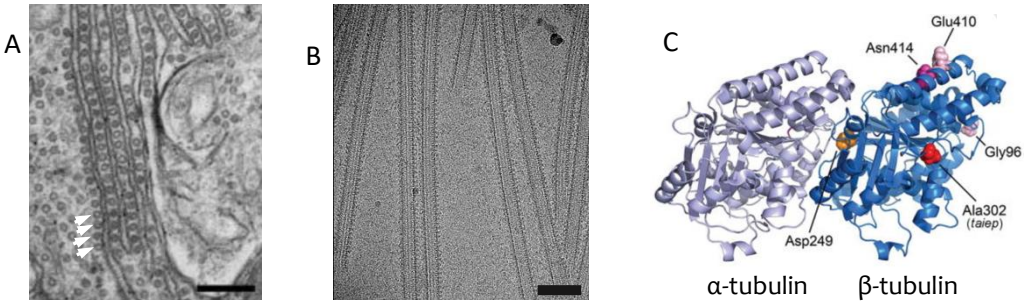

Figure 1: A) EM image of microtubules (arrows) associating with ER in H-ABC patient post-mortem brain. B) Cryo-electron microscopy image of microtubules polymerised in vitro from purified tubulin. C) A tubulin dimer showing location of H-ABC mutations in TUBB4A. Scale bars: a) 200 nm b) 100 nm. A and C adapted from[1].

References: **1)** Duncan, I. D. *et al. Ann Neurol* **81**, 690-702, (2017). **2)** Song, J. *et al. J Neurocytol* **28**, 671-683, (1999). **3)** Vemu, A. *et al. J Biol Chem* **291**, 12907-12915, (2016). **4)** Cook, A. D. *et al. J Struct Biol* **209**, 107402, (2020).

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MSci Biochemistry

Research Project -106-

Supervisor(s): Filippo Prischi	**E-mail (lead supervisor):** filippo.prischi@kcl.ac.uk
Project title: Spatiotemporal phospho-regulation of the oncoprotein YB-1	
Research area (keywords): Biochemistry, Structural Biology	
Research skills (keywords): Biochemistry, Protein expression and purification, Molecular Biology, biophysical techniques, structural biology	
Faculty/School/Department: FoLSM/Randall Centre for Cell and Molecular Biophysics	
Location: Guy's Campus	

Project description: Background. The Y-box binding protein 1 (YB-1) is a multifunctional DNA/RNA-binding protein that regulates gene expression linked to vital cellular processes, including cell proliferation, differentiation, autophagy, and stress response. After its discovery, YB-1 quite quickly became famous as it plays a crucial role in many diseases, including diabetic cardiomyopathy, carcinogenesis and viral replication (e.g., HIV and SARS-CoV-2). It has been extensively documented that YB-1 is involved in the progression of cancer, and its overexpression is regarded as a new hallmark of cancer. Specifically, in prostate cancer (PCa), YB-1 expression levels have been correlated to resistance to treatment and poor patient outcome, and in most of them the worst prognosis was associated with the increase of the phosphorylated YB-1. The p90 Ribosomal S6 Kinase (RSK) family is known to be the involved in YB-1 phosphorylation. We have recently shown that RSKs levels are also increased in PCa, and that RSKs can phosphorylate YB-1 on multiple sites, some novel and not characterised yet.

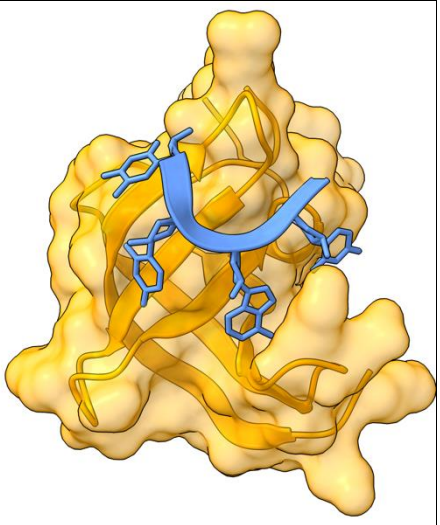

Figure 1. YB-1 (gold) in surface representation bound to RNA (blue)

Project aims. Using NMR, we were able to initially characterise the phosphorylated YB-1 and detect changes in YB-1 structure and dynamics. In this project we want to expand our study and investigate how the different phosphorylations we have discovered on YB-1 regulate its DNA/RNA binding activity. We will express and purify RSK4 and YB-1 and characterise the complex using structural biology techniques (X-Ray/SAXS/EM). We will use this YB-1-RSK4 complex to also monitor the stepwise phosphorylation events inside NMR tube to characterise spatiotemporal phospho-signaling dynamics. Finally, to identify phosphorylation-induced changes in DNA/RNA sequence specific recognition we will carry out YB1-DNA binding affinities measurements (ITC/MST/NMR). Methodologically, the student will carry out several of the following: protein expression and purification, protein crystallisation, biophysical affinity measurements (ITC/MST/NMR), NMR analysis (including sample preparation and data acquisition). Training will be provided, and NMR work will be supported by dedicated personnel.

References: (1) Cronin et al. bioRxiv (2024); (2) Chrysostomou et al. Science translational medicine, (2021); (3) Yin, et al. Cells (2022); (4) Zhang et al. Nucleic Acids Research (2020).

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MSci Biochemistry

Research Project -107-

Supervisor(s): Filippo Prischi **Konstantin Roeder**	**E-mail (lead supervisor):** filippo.prischi@kcl.ac.uk
Project title: Dissecting Internal Ribosome Entry Site elements	
Research area (keywords): Computational Biology, Biochemistry, Molecular Biology	
Research skills (keywords): Computational Biology, Biochemistry, Structural Biology (SAXS), Molecular biology, RNA/Protein expression and purification, biophysical techniques.	
Faculty/School/Department: FoLSM/Randall Centre for Cell and Molecular Biophysics	
Location: Guy's Campus	

Project description: Background. The translation of mRNAs is compromised during cell stresses that induce apoptosis. However, mRNA containing Internal Ribosome Entry Site elements (IRES) within their 5' untranslated regions (5'-UTRs) are insensitive to stress-induced repression of global translation. Under these conditions IRES-containing mRNAs binds more efficiently RNA binding proteins (RBPs), allowing continued expression of proteins required for cell survival or apoptosis. Indeed, many of the cellular mRNAs that contain IRES elements encode proteins that are involved in protection of cells from stress or, alternatively, induction of programmed cell death. Therefore, it is currently believed that cellular IRES-mediated translation plays an important role in cell-fate decisions. IRESs have complex 3D structures that often include stem loops and pseudoknots, however, no common sequence or structural motifs have been detected among cellular IRES elements. Therefore, IRES-containing cellular mRNAs must be experimentally studied to understand how RBPs bind and regulate IRES elements (Fig. 1).

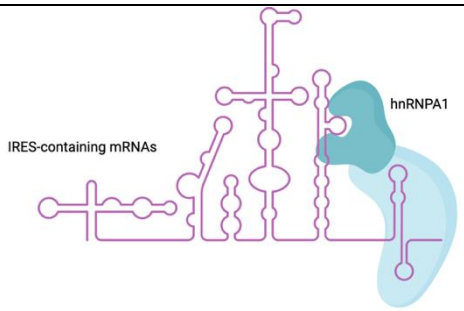


Fig. 1 Model of hnRNPA1-IRES interaction

Project aims. We have shown that in lung cancer the heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) plays a key role in disease progression by binding IRES sequences and promoting translation of, e.g., c-myc, Bcl-XL and XIAP. In this project we aim to reveal unique structural features of the IRESs, which will be correlated to hnRNPA1 recognition and interaction. This will pave the way to future drug design preventing hnRNPA1-RNA binding in cancer. We will express and purify *in vitro* large quantities of mRNA containing IRES elements, focusing primarily on the c-myc and/or XIAP IRES elements. We will then obtain experimental structural information of IRES alone and in complex with hnRNPA1 using Small Angle X-Ray Scattering (SAXS). Due to low experimental resolution, we will use computational methods to build structural models of the RNA. Using a variety of methods, including MD simulations and energy landscape explorations in combination with the HiRE coarse-grained RNA model, we will predict 3D structures for IRES sequences. These will then be refined by optimising the structure using experimental SAXS constraints. Techniques. The project is highly interdisciplinary and will involve a wide range of computational and experimental techniques, including cell culture, RNA and protein expression and purification, SAXS, and biophysical assays (including MST).

References: (1) Levengood et al. *Seminars in Cell and Developmental Biology* (2018) doi:10.1016/j.semcdb.2018.04.001.; (2) Roy *et al. Nucleic Acids Res* (2014).

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -108-

Supervisor(s): Tanya Shaw, Brian Stramer	**E-mail (lead supervisor):** tanya.shaw@kcl.ac.uk
Project title: Regulation of cell-cell adhesions in scarring/fibrosis	
Research area (keywords): skin, wound, scar, keloid, fibroblast, n-cadherin, IL6	
Research skills (keywords): cell culture, pharmacology, gene expression, protein expression and activation, protein localisation, cell signalling analysis	
Faculty/School/Department: FoLSM, School of Immunology & Microbial Sciences, Department of Inflammation Biology	
Location: Centre for Inflammation Biology & Cancer Immunology (CIBCI), 1st floor New Hunt's House, Guy's Campus	

Project description:

Pathological extracellular matrix (ECM) re-organisation is an important feature of fibrosis that influences mechanical properties of tissues (e.g. stiffness). We are studying keloid scars to interrogate mechanisms driving ECM re-organisation. We recently reported that keloid fibroblasts (KDF), compared to normal dermal fibroblasts (NDF), have a unique capacity to produce a highly aligned and bundled ECM (Figure). This is achieved through an autocrine IL-6 mechanism leading to N-cadherin-mediated cell-cell adhesions that align the cell population. This project will now investigate the cellular and molecular mechanisms controlling autocrine IL-6 and the regulation of fibrotic cell/ECM organisation through genetic and pharmacological perturbation.

Firstly, the student will learn to culture primary human fibroblasts from normal skin and keloid scars. Once the cultures are established, the student will work to identify downstream IL-6 signalling mediators that control cell and ECM alignment. Preliminary results from a phospho-kinase array revealed that KDF have elevated STAT3 activation. This project could examine effects of STAT3 inhibition on cell and ECM alignment and test other IL-6 signalling mediators by pharmacological inhibition to determine whether they are also involved in the alignment process and how they influence cell-cell adhesions.

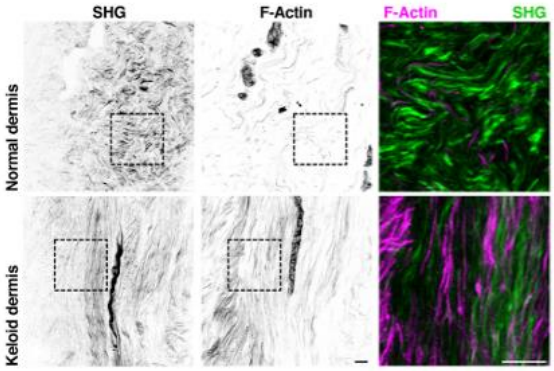


Figure. Keloid fibroblasts generate ECM anisotropy. Imaging of ECM (SHG: second harmonic generation) and F-actin in normal and keloid dermis. Note the aligned ECM and actin networks in keloid tissue. Scale bars=100µm.

References:

Kenny FN, Marcotti S, Belo de Freitas D, Drudi EM, Leech V, Bell RE, Easton JA, Diaz-de-la-Loza MC, Fleck R, Allison L, Philippeos C, Manhart A, **Shaw TJ**, Stramer BM. Autocrine IL-6 drives cell and extracellular matrix anisotropy in scar fibroblasts. Matrix Biol 2023 Nov:123:1-16. doi: 10.1016/j.matbio.2023.08.004

Barallobre-Barreiro J, Woods E, Bell RE, Easton JA, Hobbs C, Eager M, Baig F, Mackenzie Ross A, Mallipeddi R, Powell B, Soldin M, Mayr M, **Shaw TJ**. Cartilage-like composition of keloid scar ECM suggests fibroblast mis-differentiation in disease. Matrix Biology Plus 2019, 4:100016 DOI: 10.1016/j.mbplus.2019.100016.

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -109-

Supervisor(s): Gilbert Fruhwirth	**E-mail (lead supervisor):** gilbert.fruhwirth@kcl.ac.uk
Project title: **Characterization of new *in vivo* traceable and immunocompetent cancer models.**	
Research area (keywords): Cancer metastasis; imaging; cell and tissue analysis.	
Research skills (keywords): The student will perform cell culture, various in vitro assays, help establish animal tumour models, may harvest tissues, and analyse animal tissues *ex vivo* using fluorescence microscopy and/or flow cytometry. There is the option for guided *in vivo* radionuclide imaging or *ex vivo* spatial transcriptomics, if an application for the Matt Wilson fellowship were successful.	
Faculty/School/Department: Cancer and Pharmaceutical Sciences	
Location: Guy's Campus / Cancer Centre - Innovation Hub 9th floor	

Background. The human sodium iodide symporter (NIS) is a member of the superfamily of sodium/solute symporters and is physiologically implicated in cellular iodine uptake. It is expressed in only few non-thyroidal tissues such as the stomach, and at much lower levels, in mammary glands during lactation, salivary and lachrymal glands, small intestine, placenta, and testis (Portulano 2014). This restricted expression pattern together with the possibility to exploit its uptake function for in vivo imaging (via special radiotracers) has led to its development as a radionuclide reporter gene, thereby enabling non-invasive reporter gene-mediated cell tracking in mammals (Volpe 2020). We were first to design a fully functional NIS-fluorescent protein fusion (NIS-FP) to combine longitudinal multi-modal whole-body in vivo radionuclide imaging with *ex vivo* high-resolution fluorescence microscopy (Fruhwirth 2014). This permitted non-invasive and quantitative tracking of cancer cell metastasis *in vivo* (Volpe 2018), which became an invaluable tool to study the impact of therapeutics on cancers and their spread (Fruhwirth 2014; Man 2019; Volpe 2020; Maiques 2021).

For anti-cancer immunotherapy development and optimization, it is paramount to perform investigations in fully immunocompetent animals. Consequently, we engineer *in vivo* traceable cancer models that permit the systematic investigation of efficacy alone and in combination with other therapy modalities.

Project proposal. We have generated various new rodent cancer models that can be tracked in vivo exploiting rodent NIS variants. Here, we will characterize one of these models both on the cellular and tumour tissue levels. This will include growth investigations of this immunocompetent animal tumour model and downstream ex vivo analyses of important parameters such as metastatic spread, imaging reporter stability, and immune cell infiltration. Analysis methods will involve fluorescence-based methodologies including flow cytometry, immunofluorescence microscopy, and immune-histochemistry.

There is also the option to participate in guided *in vivo* SPECT/CT imaging (to quantify metastasis non-invasively) or spatial transcriptomics by Nanostring DSP GeoMX/CosMX on harvested tissues (to discover differences between primary tumour and metastases or treatment cohorts) if a Matt Wilson fellowship bid were successful.

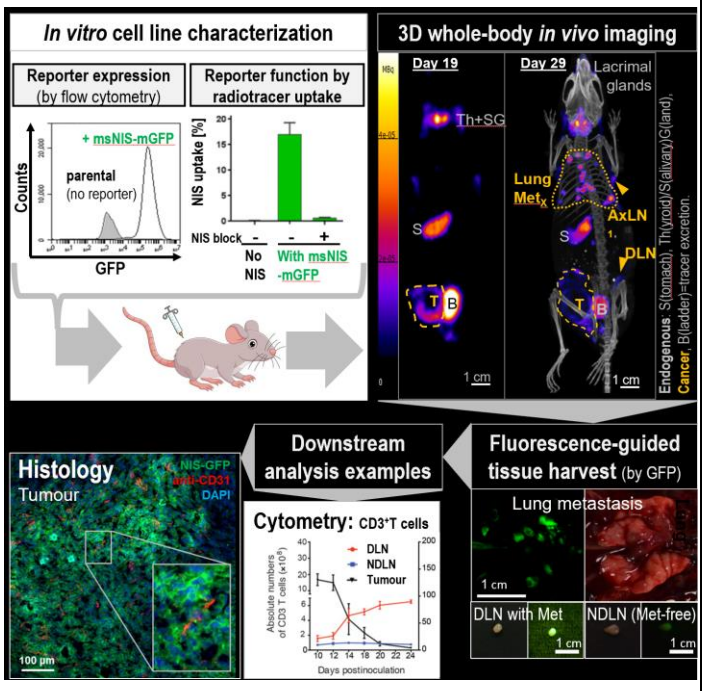


Figure (*Top left*) Example of the *in vitro* characterization of the relevant cell model in regard of the NIS-FP reporter. | (*Top right*) Typical *in vivo* imaging example of a murine orthotopic breast cancer model exploiting NIS-FP for cancer cell tracking. The left image depicts organs of endogenous NIS expression (grey inscriptions) and the growing primary tumour (yellow dashed). The right image shows the same mouse 10 days later when extensive metastasis to lymph nodes and lungs had occurred (yellow arrows/inscriptions). | (*Bottom: from right to left*) Examples of typical *ex vivo* analyses from the mouse imaged above.

References: Portulano et al Endocrin Rev (2014); Fruhwirth et al J Nucl Med (2014); Volpe et al J Vis Exp (2018); Man et al Mol Ther (2019); Volpe et al Mol Ther (2020); Maiques et al Brit J Cancer (2021).

MSc/MRes Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -110-

Supervisor(s): **Prof Christer Hogstrand (KCL), Prof Robert Price (KCL), and Dr Mark Dockrell (SWT Institute for Renal Research)**	**E-mail (lead supervisor):** **christer.hogstrand@kcl.ac.uk**

Project title: Quest for a biomarker of early Chronic Kidney Disease

Research area (keywords): diagnostics; biochemistry; diabetes; nephrology; renal failure; kidney; medicine; CTGF; CCN2

Research skills (keywords): Cell culture; Western Blot; ELISA; imaging; enzymology; bioinformatics

Faculty/School/Department: Faculty of Life Sciences and Medicine/School of Cancer and Pharmaceutical Sciences

Location: Franklin-Wilkins Building

Project description:

Diabetes is the major cause of Chronic Kidney Disease (CKD) and around 3.5M people in the UK have CKD. A sub-group of patients with CKD will progress to complete renal failure, a condition requiring life sustaining therapy of either transplantation or dialysis. Current diagnostics are insensitive and detect the disease only when irreparable damage has occurred.

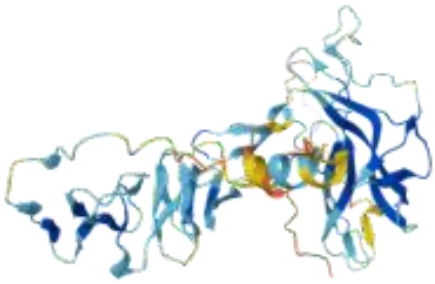


Figure 1 Alphafold model of CTGF (CCN2)

Improved screening of patients with diabetes to identify those with early CKD and likely to progress would allow earlier targeted intervention and give them an improved chance of survival of cardiovascular disease as well as potentially avoiding renal failure [1]. Connective Tissue Growth Factor (CTGF), aka CCN2, is a promising candidate biomarker for CKD as it has a key position in the pathology of the disease and is excreted in the urine [2]. Our group has recently identified that CTGF is cleaved with one of the fragments potentially mediating kidney scarring and renal failure. Consequently, the understanding how CTGF is cleaved, and the effects of the cleavage products will greatly help the understanding of the molecular basis of diabetic kidney disease [3]. Furthermore, we believe that being able to measure CTGF fragments in urine of patients will provide a sensitive and accurate test to identify patients most likely to develop progressive diabetic kidney disease.

This master's project will have three objectices, (1) to identify the enzyme which cleaves CTGF and how this links to progression on CKD, (2) to establish antibody-based assays capable of detecting full-length CTGF and CTGF fragments, and (3) analyse full-length CTGF and its fragments in urine samples from patients with different stages of CKD and healthy controls. Whether one, two, or all three of these objectives are addressed will depend on the length of the project (MRes, MSc, MSci) and interest of the student.

References: 1. Wang N, Zhang C. (2024) Recent Advances in the Management of Diabetic Kidney Disease: Slowing Progression. Int J Mol Sci. 25(6):3086. **2**. Korbut AI, et al (2024) Urinary Excretion of Biomolecules Related to Cell Cycle, Proliferation, and Autophagy in Subjects with Type 2 Diabetes and Chronic Kidney Disease. Biomedicines. 12(3):487 **3**. Kaasbøll OJ, et al. (2018) Connective tissue growth factor (CCN2) is a matricellular preproprotein controlled by proteolytic activation. J Biol Chem. 293(46):17953-17970.

Projects 111 - 113

are suitable for:

MRes students

MSci Molecular Genetics students

MSci Biochemistry students

MRes Biomedical & Molecular Sciences Research

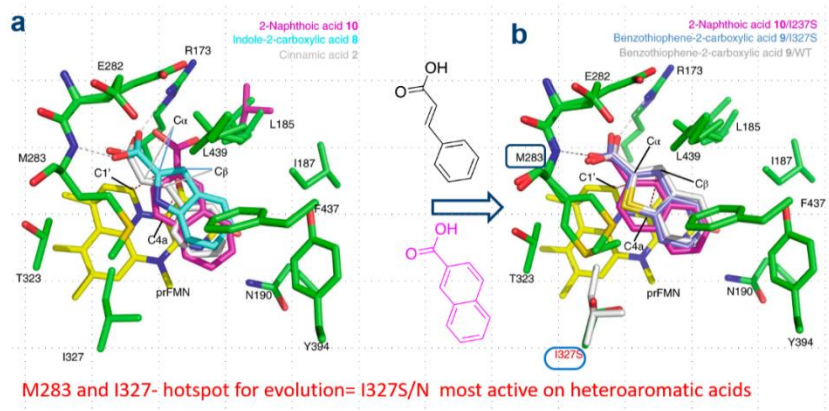
MSci Biochemistry and MSci Molecular Genetics

Research Project -111-

Supervisor(s): Dr Godwin Aleku	**E-mail (lead supervisor):** godwin.aleku@kcl.ac.uk
Project title: Finding efficient CO2-fixing enzymes for green synthesis of pharmaceuticals	
Research area (keywords): Industrial biotechnology, enzymatic synthesis, CO2-fixation	
Research skills (keywords): Enzyme discovery, Enzyme Biochemistry, molecular biology	
Faculty/School/Department: Institute of Pharmaceutical Science	
Location: Franklin Wilkins Building	

Project description: Anthropogenic recycling of CO_2 for chemical production promotes sustainable chemistry and represents one of the key strategic goals to deliver a circular and low-carbon economy.[1] Synthetic CO_2 fixation to organic compounds is an immensely attractive, sustainable synthetic approach for utilising CO_2 as a cheap, renewable C1 building block to construct complex organic frameworks from simple precursors, but progress in this area has been rather slow. Factors impeding progress are largely due to the inherent difficulties with fixing the inorganic, 'inert' CO_2 to organic molecules, the thermodynamic barrier associated with fixing CO_2 to organic compounds, and a high energy requirement for substrate activation.[2]

Using genome mining and employing 4 distinct representative query sequences from 4 different decarboxylase families, 30 putative (de)carboxylases will be carefully selected from bioinformatic analysis, cloned and expressed in *E. coli.* Using our high throughput LC-MS, each enzyme will be screened for the (de)carboxylation of 10 representatives substrates. Enzymes showing reactivity with one or more of these compounds will be further screened extensively against a structurally diverse substrate library, and the substrate scope and regioselectivity will be mapped. The most versatile enzymes will be selected for full biochemical and molecular enzymology studies and protein engineering studies of the best templates.



References:

Aleku, G. A.; Saaret, A.; Bradshaw-Allen, R. T.; Derrington, S. R.; Titchiner, G. R.; Gostimskaya, I.; Gahloth, D.; Parker, D. A.; Hay, S.; Leys, D. Enzymatic C–H Activation of Aromatic Compounds through CO 2 Fixation. *Nat. Chem. Biol.* **2020**, *16* (11), 1255–1260. https://doi.org/10.1038/s41589-020-0603

Aleku, G. A.; Roberts, G. W.; Titchiner, G. R.; Leys, D. Synthetic Enzyme-Catalyzed CO2 Fixation Reactions. *ChemSusChem* **2021**, *14* (8), 1781–1804. https://doi.org/10.1002/cssc.202100159.

MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -112-

Supervisor(s): Dr Godwin Aleku	**E-mail (lead supervisor):** godwin.aleku@kcl.ac.uk

Project title: Engineering enzymes for the synthesis of pharmaceutical drugs

Research area (keywords): Enzyme Engineering, Enzyme Biochemistry, molecular biology

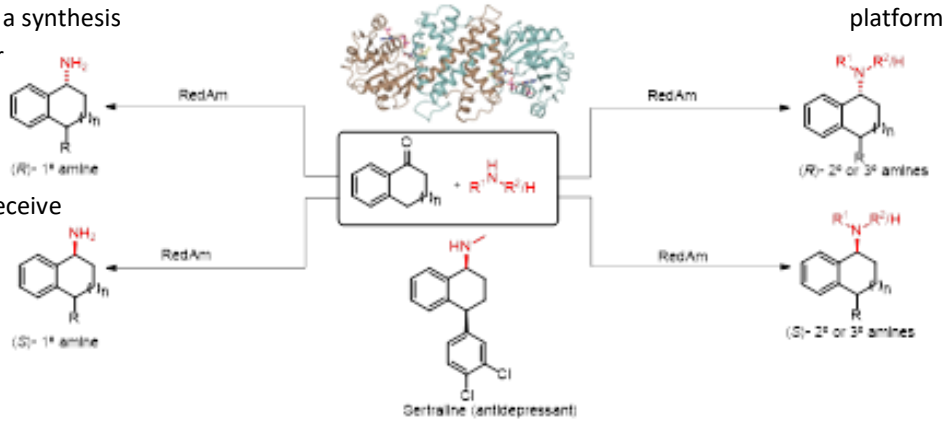
Research skills (keywords): Enzyme Engineering, Enzyme Biochemistry, molecular biology, Enzymatic synthesis

Faculty/School/Department: Institute of Pharmaceutical Science

Location: Franklin Wilkins Building

Project description: Biological systems employ enzymes to catalyse a wide range of chemical transformations to biosynthesize essential biomolecules which are often chiral. The use of enzymes (biocatalysts) for enantioselective synthesis is highly attractive for various reasons: enzymes often afford high enantioselectivity, especially towards their native substrates, and can enable green, selective, and resource-efficient industrial production. The green and sustainability credentials and the tuneable properties of biocatalysts make them attractive for exploitation in dual stereoselective synthesis Following our initial discovery an enzyme family reductive aminases (RedAms),[1] there has been significant interest in this enzyme class as they provide green, sustainable, and direct access to secondary and tertiary amines without the need to use toxic alkylating reagents. For example, RedAms have recently been applied in the synthesis of Pharmaceuticals by scientists from Pfizer[2] and GSK.[3]

In this project, the candidate will use enzyme engineering approaches to engineer RedAms for the synthesis of sertraline and other analogues. You will generate site-saturation/directed evolution libraries and perform high-throughput screening in a microtiter plate reader using an assay that has recently been developed in our group. You will perform biochemical characterisation of the best-performing hits and obtain Michaelis Menten parameters for the target substrate combination. Initially, you will chemically synthesize your target racemic product. You will develop a synthesis platform for the application of your engineered variants for the preparative synthesis of sertraline. The candidate will receive training in biocatalysis, molecular biology, protein biochemistry, enzyme engineering, and organic synthesis.



References: 1. Aleku, G. A. *et al.* A reductive aminase from *Aspergillus oryzae*. *Nat. Chem.* **9**, 961–969 (2017).

2. Kumar, R. *et al.* Biocatalytic reductive amination from discovery to commercial manufacturing applied to abrocitinib JAK1 inhibitor. *Nat. Catal.* **4**, 775–782 (2021).

MRes Biomedical & Molecular Sciences Research

MSci Biochemistry and MSci Molecular Genetics

Research Project -113-

Supervisor(s): **Jean-Marc Gallo**	**E-mail (lead supervisor):** jean-marc.gallo@kcl.ac.uk

Project title: **Altered RNA metabolism in neurodegeneration**

Research area (keywords): Neuroscience; Neurodegeneration; RNA metabolism; RNA splicing; Stem cells

Research skills (keywords): Cell culture; RNA biochemistry; RT-qPCR; western blotting

Faculty/School/Department: Institute of Psychiatry, Psychology and Neuroscience/School of Neuroscience/Department of Basic and Clinical Neuroscience

Location: Maurice Wohl Clinical Neuroscience Institute

Project description: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases with no cure. Mislocalisation of the RNA-binding protein, TDP-43, from the nucleus to the cytoplasm and aggregation are pathological hallmarks of ALS/FTD[1]. TDP-43 is involved in many aspects of RNA metabolism, including regulation of transcript stability and splicing. The N^6-methyladenosine reader and regulator of mRNA stability, YTHDF2, has been identified as a modifier of TDP-43 toxicity. YTHDF2 knockout protects neurons from TDP-43 toxicity and extends the survival of motor neurons derived from carriers of hexanucleotide repeat expansions in the *C9orf72* gene, the most common genetic cause of ALS/FTD[2]. We have conducted a transcriptome-wide TDP-43 RNA binding profiling in cells lacking YTHDF2 using individual-nucleotide resolution UV-crosslinking and immunoprecipitation (iCLIP) of protein-RNA complexes and demonstrated significant changes in the binding profile of TDP-43 in the absence of YTHDF2 (Fig. 1). TDP-43 targets regulated by YTHDF2 could therefore represent novel therapeutic entry points for ALS/FTD.

Project aims and methods. The aims of this project are: (i). To determine the role of YTHDF2 in the processing of TDP-43 targets; (ii). To determine the pathological significance of YTHDF2-modulated TDP-43 targets. The work will be conducted using haploid mouse embryonic stem cells in which the *Ythdf2* gene has been disrupted by insertional mutagenesis and are therefore perfect *Ythdf2* knockout cells (*Ythdf2* KO) and their parent cell line, AN3-12. TDP-43 targets showing a robust change in TDP-43 binding will be selected from the iCLIP analysis. Total RNA will be extracted from *Ythdf2* KO and AN3-12 cells followed by poly(A)$^+$ RNA selection. RNA will be reversed transcribed and transcript abundance will be measured by quantitative PCR using target-specific primers. Splicing will be analysed by end-point RT-PCR using pairs of primers spanning exon-exon junctions. To assess the pathological significance of the above findings, TDP-43 targets will be analysed in neurons differentiated from induced pluripotent stem cells from mutant *C9orf72* carriers or healthy controls. Western blot analysis will demonstrate whether alterations at the RNA level are reflected at the protein level.

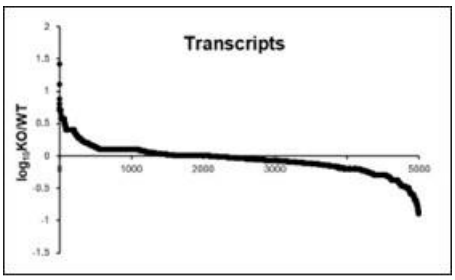


Figure 1. Comparative TDP-43 binding profile between wild-type and *Ythdf2* knockout cells.

References:

1. Neumann, M. *et al.* (2006) *Science* **314**: 130-133.
2. McMillan, M.G., N. *et al.* (2023) *Mol. Cell*. **83**: 219-236

Project 114-115

are suitable for

MSci Biochemistry students

MSci Biochemistry

Research Project -114-

Supervisor(s): **Dr Jeannine Hess, Dr Asma Akter**	**E-mail (lead supervisor):** jeannine.hess@kcl.ac.uk

Project title: Metal complexes library screening and investigation of their target engagement via CETSA for metallo-antibiotic discovery

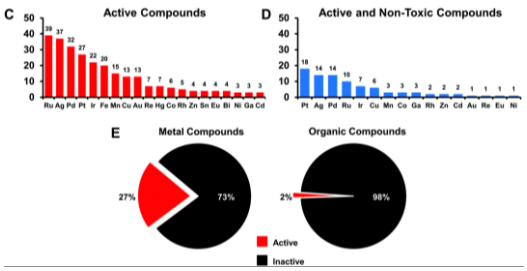
Research area (keywords): Microbiology, Metal-complexes, Antimicrobial drug discovery, Quantitative proteomics

Research skills (keywords): Microbiology techniques, Antimicrobial susceptibility testing, Biochemistry, LC-MS/MS

Faculty/School/Department: FoLSM/ Cancer & Pharmaceutical Science / AEFS

Location: Waterloo – FWB/Francis-Crick institute

Project description: The rise in antimicrobial resistance (AMR) has become a significant global health challenge, with pre-antibiotic era-level mortalities on the horizon. Recent studies found up to 1.27 million deaths attributable to bacterial AMR in 2019. Whilst the emergence of antibiotic resistance has accelerated, progress in antibiotic research has not. Given this lack of progress, as well as the low economic incentives and high failure rate of antibiotic development, we are presented with a truly dire situation. Strategic investment and novel antibiotics with innovative mechanisms of action or synergistic therapeutic combinations are therefore necessary.



Metal complexes are currently in clinical development for treating cancer, malaria and neurodegenerative diseases. By rationally designing metal complexes with diverse chemistries and combining these with cutting-edge medicinal and biological studies, we can find tailored tools to counter antibiotic-resistant infections. Frei et al. found about 10% of their metal complexes were identified as active and non-toxic, while only 0.9% of purely organic compounds showed activity with no significant toxicity. Though research describing metal complexes as antibiotics has developed significantly over the past five decades, there is still much work to do before we see many of these compounds entering the clinic as drugs. Compared with the plethora of work on organic-based drug molecules, metal-based drug development is still in its early stages, and normalising the development of metal-based drugs has been a slow process.

The goal of this project is to discover novel metal complexes with antimicrobial activity against clinically relevant pathogens. The student will aim to:

1. Screen a metal complex library from Hess lab against ESCAPE (bacteria) and clinically relevant fungi.
2. Determine MIC and MBC of initial metal complex hits.
3. Determine *in vitro* toxicity and haemolysis assay for potential metalloantibiotics.
4. Perform cellular thermal shift assay (CETSA) for promising cellular targets identifications.
5. *In vivo* toxicity of these metallo-antibiotics in greater wax moth *Galleria mellonella* (if time allows).

References: **(1)** Frei, A., Verderosa, A.D., Elliott, A.G. et al. Metals to combat antimicrobial resistance. Nat Rev Chem 7, 202–224 (2023). doi: 10.1038/s41570-023-00463-4. **(2)** Waters JE, Stevens-Cullinane L, Siebenmann L, **Hess J**. Recent advances in the development of metal complexes as antibacterial agents with metal-specific modes of action. Curr Opin Microbiol.75:102347 (2023). doi: 10.1016/j.mib.2023.102347. **(3)** Rubbiani R, Weil T, Tocci N, Mastrobuoni L, Jeger S, Moretto M, Ng J, Lin Y, **Hess J**, et al. *In vivo* active organometallic-containing antimycotic agents. RSC Chem Biol. 2(4):1263-1273 (2021). doi: 10.1039/d1cb00123j. **(4)** Frei A, Zuegg J, Elliott AG, et al. Metal complexes as a promising source for new antibiotics. Chem Sci. 2627-2639 (2020). doi: 10.1039/c9sc06460e. **(5)** Tu Y, Tan L, Tao H, et. al. CETSA and thermal proteome profiling strategies for target identification and drug discovery of natural products. Phytomedicine. 2023 Jul 25;116:154862. doi: 10.1016/j.phymed.2023.154862.

MSci Biochemistry

Research Project -115-

Supervisor(s): Prof Michael Antoniou	**E-mail (lead supervisor):** michael.antoniou@kcl.ac.uk
Project title: The role of the CTCF transcription factor in Rps3 ubiquitous chromatin opening element (UCOE) function	
Research area (keywords): housekeeping genes; regulation of gene expression; transcription factors	
Research skills (keywords): mammalian cell tissue culture; cell transfection; ELISA; DNA extraction; RT-qPCR	
Faculty/School/Department: Department of Medical and Molecular Genetics, BMBS, FoLSM	
Location: 7th Floor Guy's Tower	

Project description: Housekeeping genes (HKGs) encode for proteins, which all cells need and thus constitute the physiological foundation of life. In addition, regions from some HKGs have been found to possess a ubiquitous chromatin opening element (UCOE®) function. UCOEs confer upon transgenes stable and reproducible expression and have been exploited in expression vectors for biotechnological applications (therapeutic protein biomanufacture, gene therapy). However, the regulation of HKG expression has attracted little attention and is poorly understood. Bioinformatics and functional analyses in tissue culture cells in the host laboratory has begun to reveal that regulation of HKGs is highly complex involving clusters of different types of transcription factors (TFs) and co-factors performing different roles (chromatin remodelling, transcription activation). It has been found that TF binding sites can be located not just at HKG promoters but also at distant locations. One TF found to be associated with the promoter region of all HKGs is CTCF, which suggests it performs a crucial role in regulating expression. CTCF can self-interact and thus bring distantly located genomic regions into proximity. Murine *Rps3*, an HKG possessing potent UCOE function, possesses two CTCF binding sites, one in the promoter region and another over 1000bp 3' of the promoter amongst a cluster of other TF sites. Host lab analysis in tissue culture cells has shown that the 3' cluster of TFs is essential for full Rps3 UCOE function.

Aims: We hypothesise that CTCF is responsible for bringing together the promoter-associated and 3' cluster of TFs to allow full Rps3 UCOE function. The aim of this project is to functionally test this hypothesis.

Methods: The student will be provided with Rps3 UCOE test gene constructs that have had the promoter and 3' TF cluster CTCF sites mutated either singly or in combination. Test gene constructs expressing Etanercept protein as a reporter will be stably transfected into CHOZN-K1 cells in parallel with wild-type Rps3 UCOE and UCOE-less controls. Etanercept protein production will be measured by enzyme linked immunosorbent assay (ELISA). Expression will be compared between the CTCF mutant constructs and the wild-type Rps3 and UCOE-less controls. Assays will be conducted under industry standard continuous fed-batch cell culture conditions over 2-3 weeks to assess both level and stability of expression. Real time-qPCR (RT-qPCR) will be used to determine average transgene copy number per transfected cell against which transgene expression will be normalised.

References: Neville JJ et al. (2017) Ubiquitous Chromatin-opening Elements (UCOEs): Applications in biomanufacturing and gene therapy. Biotechnol Adv. **35**: 557-564; Joshi CJ et al. (2022) What are housekeeping genes? *PLoS Comput Biol*. **18**: e1010295

Project 116-119

are suitable for:

MRes students

MRes Biomedical & Molecular Sciences Research

Research Project -116-

Supervisor(s): **Prof Marika Charalambous**	**E-mail (lead supervisor):** marika.charalambous@kcl.ac.uk
Project title: Fetal control of the maternal brain in pregnancy	
Research area (keywords): Pregnancy, metabolism, neuroendocrine, fetal growth	
Research skills (keywords): Histology, immunofluorescence, mouse models	
Faculty/School/Department: FoLSM/BMBS/MMG	
Location: Guy's Tower/The Francis Crick Institute	

Project description:

The maternal metabolic profile undergoes profound shifts over the course of a normal pregnancy. Early adaptations promote the storage of nutrients in maternal tissues, while net catabolic pathways in late gestation modulate the allocation of nutrients between mother and fetus. Many of these adaptations are driven by placental hormones that promote the transfer of nutritional fuel to the growing fetus.

The system-wide maternal adaptations suggest that placental hormones might regulate the central neuronal circuitry that regulate whole-body energy homeostasis. Outside of pregnancy anatomically distinct neuronal populations in the hypothalamus sense hormones and nutrients in the blood and signal to other neurons to ultimately adjust food intake and energy expenditure. Despite some evidence of placental hormones targeting their receptors in the maternal hypothalamus, our mechanistic understanding of hormone-receptor signalling, and the specific neuronal pathways they target, are poorly understood.

We have generated a single-cell RNA sequencing (scRNA-seq) dataset of the hypothalamus from virgin and late-pregnant mice. Comparison of cell-type specific transcriptomes uncovered numerous differentially expressed genes across neuronal and non-neuronal hypothalamic cell-types in pregnancy. This project will combine scRNA-seq data analyses with immunohistochemistry experiments to select and validate candidate hormone-driven pathways that are changed in the maternal hypothalamus.

References:

1) Napso, T.; Yong, H. E. J.; Lopez-Tello, J.; Sferruzzi-Perri, A. N. J. Neuroendocrinol. 2010, 22 (7), 805–817. *https://doi.org/10.1111/j.1365-2826.2010.02017.x*.
2) Clarke, G. S.; Gatford, K. L.; Young, R. L.; Grattan, D. R.; Ladyman, S. R.; Page, A. J. Obes. Silver Spring Md 2021, 29 (11), 1813– 1824. https://doi.org/10.1002/oby.23224
3) Khant Aung, Z.; Grattan, D. R.; Ladyman, S. R. Mol. Cell. Endocrinol. 2020, 516, 110933. *https://doi.org/10.1016/j.mce.2020.110933*.
4) Augustine, R. A.; Knowles, P. J.; Khant Aung, Z.; Grattan, D. R.; Ladyman, S. R. J. Neuroendocrinol. 2019, 31 (9), e12702. https://doi.org/10.1111/jne.12702.
5) Teixeira, P. D. S.; Couto, G. C.; Furigo, I. C.; List, E. O.; Kopchick, J. J.; Donato, J. Am. J. Physiol.-Endocrinol. Metab. 2019, 317 (5), E925–E940. https://doi.org/10.1152/ajpendo.00229.2019

MRes Biomedical & Molecular Sciences Research

Research Project -117-

Supervisor(s): Charlotte Odendall	**E-mail (lead supervisor):** charlotte.odendall@kcl.ac.uk

Project title: Role of Interferon-Stimulated Genes in Modulating *Salmonella* Replication and Dormancy in Epithelial Cells

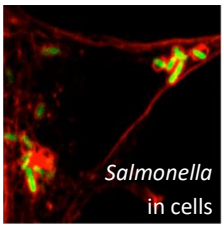
Research area (keywords): Host-pathogen interactions

Research skills (keywords): Microscopy, Flow Cytometry, CRISPR/Cas, Cellular microbiology

Faculty/School/Department: School of Immunobiology and Microbial Sciences, Infectious Diseases

Location: Guy's Hospital, Borough Wing, 3rd Floor

Project description: *Salmonella enterica* serovar Typhimurium is a leading cause of bacterial gastroenteritis, responsible for millions of infections globally each year. *Salmonella* targets the intestinal epithelium and uses specialised virulence factors to invade and multiply within cells. In epithelial cells, *Salmonella* replicates within a membrane bound *Salmonella* containing vacuole (SCV) or escapes into the cytosol, where it can replicate more efficiently.



Interferons (IFNs), particularly Type I (IFN-α, IFN-β) and Type III (IFN-λ), play a crucial role in shaping the host immune response to infections. Upon detection of a pathogen, IFNs are produced and stimulate the expression of a broad range of interferon-stimulated genes (ISGs), which create an antimicrobial environment[1, 2]. While the antiviral functions of IFNs and ISGs are well-established, their role in bacterial infections, particularly in controlling intracellular pathogens like *Salmonella*, remains less clear. Our preliminary work has shown that IFNs can restrict *Salmonella* replication, but the specific ISGs involved in this process are poorly understood.

We have found that IFN treatment limits *Salmonella*'s ability to escape the SCV and enter the cytosol, which limits bacterial expansion. Instead, IFN appears to push the bacteria towards a dormant state in vacuoles. Further, we have performed a high-throughput screen of 500 human ISGs and identified several candidates that modulate *Salmonella* infection. Five ISGs—CALHM6, EHD4, LAMP3, VAMP5, and NCOA7—were found to significantly reduce bacterial replication and promote bacterial dormancy. These ISGs, localised on endosomes, are poised to modulate vesicle trafficking and lysosomal function, potentially maintaining SCV stability or promoting bacterial dormancy.

This project will focus on investigating the specific roles of these five ISGs in controlling *Salmonella* replication and dormancy. The aims of this study are to:

Aim 1: Construct genetic knockout of these ISGs. CRISPR/Cas will be employed to genetically delete CALHM6, EHD4, LAMP3, VAMP5, and NCOA7 in epithelial cells and macrophages.

Aim 2: Determine the importance of these genes in controlling *Salmonella* infection. Infection assays will be carried out in these cells. The ability of each of these genes to control *Salmonella* will be assessed by microscopy, flow cytometry and plating of live bacteria.

Aim 3: Determine the impact of these genes on *Salmonella* subcellular localisation. We will use elaborate reporters, flow cytometry and powerful microscopy to determine how these genes affect *Salmonella* escape into the cytosol or dormancy.

References:

1- Alphonse N, Dickenson RE, Odendall C (2021) Interferons: Tug of War Between Bacteria and Their Host. Front Cell Infect Mi 11:624094. https://doi.org/10.3389/fcimb.2021.624094

2- Alphonse N, Wanford JJ, […], Odendall C (2022) A family of conserved bacterial virulence factors dampens interferon responses by blocking calcium signaling. Cell. https://doi.org/10.1016/j.cell.2022.04.028

MRes Biomedical & Molecular Sciences Research

Research Project -118-

Supervisor(s): Setsuko Sahara	**E-mail (lead supervisor):** setsuko.sahara@kcl.ac.uk

Project title: Molecular controls of sex-biased gene expression in brain development

Research area (keywords): Stem cells, X-inactivation, Cortical development, Neurogenesis and Gliogenesis

Research skills (keywords): human and mouse ES/iPS cell culture, Molecular biology, FISH, Bioinformatics, CRISPR editing, imaging and analysis

Faculty/School/Department: Centre for Developmental Neurobiology

Location: CDN functional genomics centre, New Hunt House and Hodgkin Building, Guys Campus

Project description:

X chromosome inactivation (XCI) is an epigenetic mechanism that equalises X-linked gene dosage between males and females. Whilst XCI was previously believed to persist throughout life, it has been observed that certain genes either consistently remain active or become reactivated in a lineage-specific, developmental, or age-specific manner. The reactivation of these 'facultative escapees' is speculated to contribute to female-specific disease susceptibility and sexual dimorphisms, yet the regulatory mechanisms behind this phenomenon remain unclear.

Recently, we discovered that several crucial XCI regulators exhibit temporal changes in isoform expression during corticogenesis. These changes may play a role in triggering the reactivation of escapee genes.

To elucidate the impact of developmentally regulated XCI escapee genes on development, the student may choose from the following projects:

1. Establishing an ES cell-based in vitro brain development model to investigate the trajectories of sex-biased allele-specific gene expression.
2. Normalising the erosion of X-chromosome inactivation in human iPS cells to model sex-biased gene expression during human brain development.

Throughout the project, the student will learn stem cell manipulation, culturing, and various molecular and gene-editing techniques. This project is well-suited for students interested in stem cell fate decisions, sex-biased gene expression, CRISPR-based technologies, and culturing mouse ES and human iPS cells. Whilst prior experience and knowledge in cell culture and molecular biology are not essential, they are preferable.

References: 1. Jacobson, E. C., Pandya-Jones, A. & Plath, K. A *Current Opinion in Genetics & Development* 75, 101927 (2022). 2. Loda, A., Collombet, S. & Heard, E. *Nat Rev Mol Cell Biol* 23, 231–249 (2022). 3. Lodato, S., Shetty, A. S. & Arlotta, P. *Trends in Neurosciences* doi:10.1016/j.tins.2014.11.003. 4. Colognori, D., Trinidad, M. & Doudna, J. A *Nat Biotechnol* 1–9 (2023) doi:10.1038/s41587-022-01649-9.5. Choi, H. M. T. *et al.* T. *Development* 145, dev165753 (2018)

MRes Biomedical & Molecular Sciences Research

Research Project -119-

Supervisor(s): Alex Dyson	**E-mail (lead supervisor):** alex.dyson@kcl.ac.uk

Project title: Development of slow-release selenides for treatment of redox-based pathologies

Research area (keywords): Pharmacology, biochemistry, drug development

Research skills (keywords): Cell culture; Biochemistry techniques e.g. enzyme assays, ELISAs; Physiological techniques e.g. oxygen consumption assays, mitochondrial function

Faculty/School/Department: FoLSM, School Cancer and Pharmaceutical Sciences

Location: Waterloo, Franklin Wilkins Building

A common biological feature of the chalcogens (oxygen, sulfur, selenium) is their ability to modulate metabolism, and this has underlined their potential utility as therapeutics that can treat redox-based pathologies. A landmark study showed the ability of hydrogen sulfide (H_2S) to induce suspended animation in mice [1]. A spectacular upsurge in the development of slow release sulfide 'donors' to avert oxidative pathologies followed, with compounds that exhibited both controllable sulfide release (to prevent toxicity) and targeted administration (to prevent off-target effects). More recently, both the gaseous [2] and basic salt [3] forms of hydrogen selenide (H_2Se) have demonstrated similar effects to hydrogen sulfide, and this includes lifespan extension in *C.elegans* (unpublished observations).

This project aims to characterise a 'first-in-class' slow-release selenide donor, specifically examining its pharmacology and effects on oxidative metabolism and redox status. It is anticipated this project will generate key pilot data, further elucidate mechanism(s) of actio and support a drug-development opportunity.

The project will require cell culture techniques, with cultured cells derived from primary cell lines exposed to either sodium hydroselenide (a selenide generator used as a positive control) and tetraselenotungstate (a putative slow-release selenide donor in the selenometallate drug class). Example assays include cytotoxicity testing in cell lines, oxygen consumption in cells and *ex vivo* tissues and mitochondrial enzyme activities assessed using spectrophotometric an optode luminescence-based techniques. Advanced techniques such as confocal microscopy wi be used to elucidate mechanisms of action along with (subject to further funding) transcriptomic analyses.

Lab experience is preferable although the student will be provided with full training in all techniques, as required.

References:

[1] Blackstone (2005) Science; 308:518
[2] Iwata (2015) Crit Care Med; 43:1361
[3] Samra (2021) Int J Mol Sci; 22:3258

Projects 120-122

are suitable for:

MSci Molecular Genetics students

MSci Biochemistry students

MSci Biochemistry and MSci Molecular Genetics

Research Project -120-

Supervisor(s): Deepak Srivastava	**E-mail (lead supervisor):** deepak.srivastava@kcl.ac.uk

Project title:

Research area (keywords): Stem cells, RNA splicing, synapse, miRNA, neurodevelopment, neurodevelopmental conditions

Research skills (keywords): molecular biology, stem cell culture, microscopy, imaging, gene editing (CRIPSR-Cas9), organoid culture

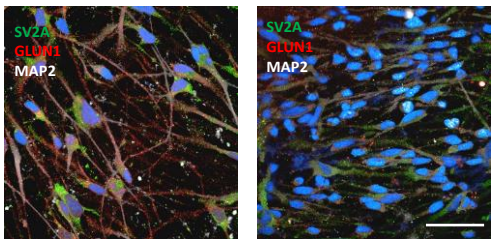
Faculty/School/Department: IoPPN/Neuroscience/Basic and Clinical Neuroscience

Location: Denmark Hill

Project description:

This study aims to explore the **regulatory effect of microRNAs *Neurexin 1* (*NRXN1*) isoform expression and its impact on synaptic dysfunction within neuropsychiatric disorders**. As multiple lines of evidence support a key role for key role for abnormal synaptic connectivity in disorders including schizophrenia, developing novel therapeutic approaches to target and modulate is of great interest. Mutations in *NRNX1* are strongly implicated in the pathogenesis of neuropsychiatric disorders such as schizophrenia, and the gene encodes for a synaptic adhesion protein known to play a critical role in regulating synaptic function. Preliminary data from our group demonstrates that specific miRNAs regulate *NRXN1* isoform expression, and rectifies abnormal synaptic function in human-induced pluripotent stem cell (hiPSC)-derived neurons generated from individuals with *NRXN1*-mutations. We **hypothesise that inhibiting or mimicking the function of specific mircoRNAs regulates *NRXN1* isoform expression, and thereby impact synaptic function**. To test this hypothesis, the project will aim to address the following objectives:

1. To determine whether specific microRNAs directly interact with the *NRXN1* gene – here we will use a reporter system to see if specific microRNAs directly bind to regions within the gene.
2. To elucidate the impact inhibiting or mimicking specific microRNA expression has on *NRXN1* isoform expression (directly or indirectly) – here we will inhibit or drive the expression of specific microRNAs and subsequently look at the expression pattern of different *NRXN1* isoforms in neurons and organoids generated from hiPSCs.



References: Flaherty E, et al., Nat Genet. 2019 Dec;51(12):1679-1690;
Fernando MB, et al., bioRxiv [Preprint]. 2023 Dec 23:2023.10.28.564543;
Liakath-Ali K, Südhof TC. Front Mol Neurosci. 2021 Mar 9;14:659681;
Dutan et al; bioRxiv [Preprint]. 2024 Aug 26:609494.

MSci Biochemistry and MSci Molecular Genetics

Research Project -121-

Supervisor(s): Dr Virve Enne **Dr Daniel Ward**	**E-mail (lead supervisor):** virve.enne@kcl.ac.uk

Project title: Investigating the utility of nanopore respiratory metagenomic sequencing for generating whole genome pathogen sequences

Research area (keywords): metagenomics, microbiology, genomics, bioinformatics

Research skills (keywords): molecular biology, nanopore sequencing, bacterial culture and susceptibility testing, bioinformatics

Faculty/School/Department: Department of Infectious Diseases

Location: CIDR, 5th Floor, St. Thomas's Hospital

Project description:

The Centre for Infectious Disease and Diagnostic research (CIDR) at based at St. Thomas's hospital, is a cutting-edge interdisciplinary group of scientists, clinicians and bioinformaticians working on implementing shotgun metagenomics (RMg) in the clinic. Specifically, the team work with Oxford Nanopore Technologies (ONT) sequencing devices, implementing laboratory and bioinformatic pipelines designed to diagnose serious respiratory and other infections within 1 day.

At CIDR our ongoing objective is to translate experimental diagnostic techniques into clinical use. The student will assist with development and evaluation efforts of the CIDR RMg workflow. Specifically, in this project the student will lead an investigation into the capability of nanopore shotgun respiratory metagenomics to generate bacterial whole genome sequences from complex respiratory clinical samples. Bacterial whole genome sequences are important for understanding antimicrobial resistance, pathogen transmission networks and potential virulence. The student will isolate and identify bacteria from metagenomic samples using traditional microbiological techniques. Employing molecular techniques, the student will prepare genomic libraries for sequencing on ONT devices. With a significant bioinformatics component, this project requires the analysis of genomic data, assembling bacterial genomes and a creative investigation into downstream applications such as AMR and virulence prediction. Finally, they will perform a comparative analysis of the data generated. The student will have the opportunity to co-author a manuscript based on their results. Students will receive the training required for all techniques. Prior experience in a field of data science is desirable as the bioinformatics research component is expected to be mostly self-driven.

References:

JD Edgeworth. 2023. Respiratory metagenomics. *Curr Opin Infect Dis* **36:**115-123

T Charalampous, A Alcolea-Medina, LB Snell *et al*. 2024. Routine metagenomics service for ICU patients with respiratory infection. *Am J Respir Crit Care Med* **15:**164-174.

MSci Biochemistry and MSci Molecular Genetics

Research Project -122-

Supervisor(s): Dr Joanna Jacków, Dr. Sheila McSweeney	**E-mail (lead supervisor): joanna.jackow@kcl.ac.uk**

Project title: Investigation of lipid-mediated mechanisms causing cutaneous photosensitivity in solar urticaria

Research area (keywords): Molecular biology, photobiology, skin biology

Research skills (keywords): Experimental design, fibroblast isolation, cell culture, polymerase chain reaction, flow cytometry, fluorescence microscopy, immunohistochemistry, short interfering RNA and gene knockdown.

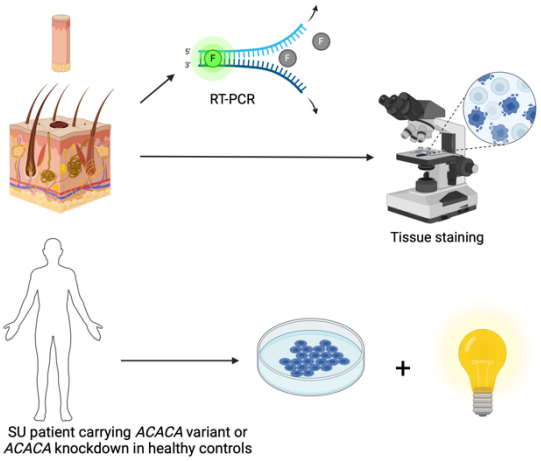
Faculty/School/Department: St. John's Institute of Dermatology, Faculty of Life Sciences and Medicine

Location: St. John's Institute of Dermatology, 9th Floor Tower Wing, Guy's Hospital, Great Maze Pond, SE1 9RT

Project description:

Solar urticaria (SU) is a rare photosensitive skin disease characterised by inappropriate cutaneous mast cell activation following exposure to solar radiation. Patients with SU develop immediate wheals ("hives") with exposure to ultraviolet A or visible light and in severe cases, anaphylactic shock. Unsurprisingly, affected individuals report a profound impact on their quality of life (**Botto *et al.,* 2008**). Previous work in our group has identified significant enrichment of rare, heterozygous, pathogenic, missense variants in *ACACA* amongst SU patients (**McSweeney *et al.,* unpublished data**). *ACACA* encodes acetyl-coenzyme A carboxylase alpha, a ubiquitously expressed cytosolic enzyme that catalyses the first, rate-limiting step of *de novo* lipogenesis. Bulk RNA sequencing has also demonstrated altered expression of lipid metabolism pathways in SU skin compared to healthy controls, as well as pathways known to counteract oxidant stress from reactive oxygen species (ROS), e.g., glutathione conjugation (**McSweeney *et al.,* unpublished data**). As ultraviolet A and visible light interact with skin to produce ROS and are also triggering wavebands in SU, these findings imply that lipid-related disease mechanisms in SU could relate to lipid peroxidation by ROS (**Kammeyer and Luiten, 2015**).

This project aims to validate and functionally interrogate SU pathogenic mechanisms mediated by lipid peroxidation. It could extend over 6 or 9 months and will benefit from existing skin samples already stored in a large, SU-specific bioresource at St. John's Institute of Dermatology. During the project, the student will: **(1) Validate expression of genes whose products promote lipid peroxidation in SU skin compared to healthy controls via RT-PCR and immunohistochemical/immunofluorescent staining; and (2) Use *in vitro* cell culture models to interrogate the relationship between variants in *ACACA* carried by SU patients, its expression, and cellular susceptibility to lipid peroxidation pre- and post-exposure to a physiologically relevant dose of ultraviolet A.** Findings from these experiments could provide exciting mechanistic insights into SU and have wider implications for understanding of photosensitive skin disease. **I would recommend this project to any student interested in translational and clinical research, especially in skin or other epithelial tissues.**



Techniques and skills involved in this project: Experimental design, fibroblast isolation, cell culture, polymerase chain reaction, flow cytometry, fluorescence microscopy, immunohistochemistry, short interfering RNA and gene knockdown.

References: Kammeyer A, Luiten RM. Oxidation events and skin aging. Ageing Res Rev. 2015; 21:16-29. Botto NC, Warshaw EM. Solar urticaria. J Am Acad Dermatol. 2008;59(6):909-20.

Project 123

is suitable for:

MSc students

MSci Biochemistry students

MSc Biomedical & Molecular Sciences Research

MSci Biochemistry

Research Project -123-

Supervisor(s): Dr. M. Teresa Martín Monreal, Dr. Joanna Jacków, Dr. Panicos Shangaris.

E-mail (lead supervisor): teresa.monreal@kcl.ac.uk

Project title: Placenta in a dish – study of placental spheroid formation and their immune landscape

Research area (keywords): Spheroids, placental immunology, pregnancy adverse outcomes, immunoregulation

Research skills (keywords): Experimental design, tissue digestion, cell culture, spheroid formation, flow cytometry, fluorescence microscopy.

Faculty/School/Department: Faculty of Life Sciences and Medicine, School of Immunology & Microbial Sciences, Peter Gorer Department of Immunobiology

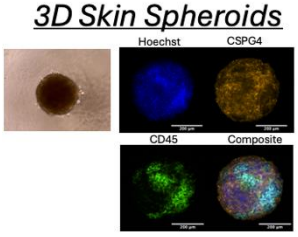
Location: Peter Gorer Department of Immunobiology, 5th Floor Bermondsey/Southwark Wing, Guy's Hospital, Great Maze Pond, SE1 9RT

Project description:

Our research is based on the study of the maternal immune system during pregnancy and its role in pregnancy adverse outcomes (PAOs) with a specific focus on regulatory T cells (Tregs). Previous studies have linked lower circulating levels of Tregs with PAOs such as pre-term labour, pre-eclampsia, and gestational diabetes[1–5]; however, the exact mechanisms by which Tregs are involved in these conditions remain obscure. An accessible source to study the maternal immune cells is the peripheral blood, and our group has collected, to this date, more than 1500 samples of peripheral blood mononuclear cells from pregnant individuals. Although informative and easily accessible, there are nuances and differences in the circulating Treg subpopulations compared to those that are tissue-resident. Thus, the group has also developed a protocol to obtain single-cell suspensions from the maternal side of the placenta. These suspensions contain a mix of cells including trophoblast and immune tissue-resident cells. Obtaining single-cell solutions from placentas allows us to characterise the immune (and non-immune) landscape in PAOs, in particular the Treg subpopulation picture, and compare it to the circulating fraction. Additionally, it opens the door to study the Treg activity further, in a "close-to-in-vivo" set up using placental spheroids. To this date, spheroid models of the human placenta are based on a trophoblast composition but lack the complexity of the immune cell presence. In our lab, we aim to build on top of those models by using a more holistic approach, similar to what has been published using tumour and tonsil single cell solutions[6,7]. Taking advantage of the expertise in 3D tissue models of the Jacków lab (image panel), we aim to create placental spheroids derived from the aggregation of heterogenous single-cell placenta suspensions.

This is a 6-month project. During this time the student will work on the following steps:

1. Testing the optimal number of cells and conditions needed to create different types of placenta spheroids: derived from single-cell placental solutions, trophoblast cell lines (BeWo cells), and/or primary trophoblast.
2. Assess the migration of immune cells (derived from placenta or peripheral blood) into placental spheroids lacking those.
3. Characterise the spheroid composition by means of fluorescence microscopy and/or flow cytometry.



This project will be ideal for a dedicated student with a strong interest in immunology and cutting-edge techniques regarding 3D tissue modelling. The findings of this project will allow us to better study the Treg function in the placenta by mimicking in a dish a process that is cumbersome to study in vivo (without resorting to animal models) for obvious ethical reasons. Techniques and skills involved in this project: tissue dissociation (placenta), spheroid formation, flow cytometry, cell culture, fluorescence microscopy and imaging.

References:

1. Koucký, M. *et al.* . *J. Reprod. Immunol.* **106**, 110–117 (2014). **2.** Gomez-Lopez, N. *et al. Cell Rep.* 32, (2020). **3.** Sasaki, Y. *et al. Clin. Exp. Immunol.* **149**, 139–145 (2007). **4.** Green, S. *et al. Front. Immunol.* 12, 1–13 (2021). **5.** Arain, H. *et al. Front. Immunol.* 14, (2023). **6.** Hofmann, S., Cohen-Harazi, R., Maizels, Y. & Koman, I. *Transl. Cancer Res.* 11, 134–147 (2022). **7.** Wagar, L. E. *et al. Nat. Med.* 27, 125–135 2021).

Projects 124

is suitable for

MSc students

MSci Molecular Genetics students

MSc Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -124-

Supervisor(s): Dr Julia Sarah El-Sayed Moustafa and Professor Kerrin Small	**E-mail (lead supervisor):** julia.el-sayed_moustafa@kcl.ac.uk

Project title: Identification of molecular signatures of type 2 diabetes using multi-tissue gene expression data in the TwinsUK cohort

Research area (keywords): Computational genomics, genetics, multi-tissue gene expression, cardiometabolic disease, polygenic scores

Research skills (keywords): Statistical analysis using R, data manipulation and programming, bash shell scripting

Faculty/School/Department: School of Life Course and Population Sciences

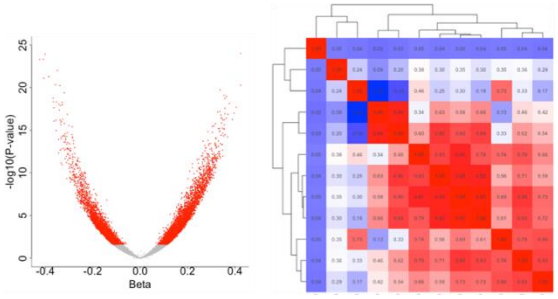
Location: Department of Twin Research and Genetic Epidemiology, King's College London, 3rd floor south wing, St Thomas Hospital, Westminster

Project description:

Cardiometabolic diseases, including type 2 diabetes, cardiovascular disease, and obesity, are among the leading causes of morbidity and mortality worldwide. Type 2 diabetes affects over 537 million people worldwide, and contributes strongly to the incidence of cardiovascular disease and kidney failure, yet significant gaps remain in our understanding of the molecular factors underlying its development and progression.

TwinsUK is a longitudinal population study of approximately 15,000 twins which has been running for over 30 years, with a wealth of cross-sectional and longitudinal multi-omic, clinical, and biochemical data collected, including a unique multi-tissue gene expression dataset.

This project will explore the molecular underpinnings of type 2 diabetes using multi-tissue gene expression data in the TwinsUK cohort to assess the impact of genetic susceptibility to type 2 diabetes on gene expression levels. This is a fully computational project where the student will gain experience in the analysis and integration of RNA-Seq gene expression data, genome-wide genotyping data, polygenic scores, and phenotypic data, gaining experience in data manipulation and statistical analyses using R and shell scripting. Students undertaking this project would join the Regulatory Genomics group within the Department of Twin Research and Genetic Epidemiology, gaining experience in computational genomics in a very active and welcoming research environment.



References:

Type 2 diabetes: Suzuki et al (2024), Nature, 627, 347–357.

TwinsUK cohort: Moayyeri et al (2013). Twin Research and Human Genetics, 16(1), 144-149.

Multi-tissue gene expression data in TwinsUK: Buil *et al*, (2015). Nature Genetics, 47(1), 88-91; Grundberg *et al*, (2012), Nature Genetics, 44(10),1084-1089.

Project 125

is suitable for:

MRes students

MSci Molecular Genetics students

MRes Biomedical & Molecular Sciences Research

MSci Molecular Genetics

Research Project -125-

Supervisor(s): **Cathy Shanahan**	**E-mail (lead supervisor):** cathy.shanahan@kcl.ac.uk

Project title: Understanding the epigenetic regulation of vascular calcification

Research area (keywords): vascular, calcification, ageing, epigenetics

Research skills (keywords): Tissue culture, siRNA, Western blot, ChIP-assays, cut and run, bioinformatics of ChiP-seq data and single-cell sequencing data

Faculty/School/Department: School of Cardiovascular and Metabolic Medicine and Sciences, FoLSM

Location: James Black Centre, Denmark Hill

Project description:

Ageing is the strongest risk factor for the development of vascular calcification, a detrimental, prevalent pathology for which there is no treatment. Calcification is caused by vascular smooth muscle cell (VSMC) phenotypic change from a contractile cell to a 'synthetic' modified cell. In ageing and calcification VSMCs convert to osteogenic-like cells and, in a process similar to bone formation, these cells orchestrate the calcification process[1].

We are interested to understand the factors that initiate this change to an osteogenic phenotype and in particular the role of epigenetics, as epigenetic change is influenced by the environment. Our data shows that the epigenetic signature of VSMCs is dynamically rewritten over the calcification time-course in response to various stresses and we have identified the Bromodomain (Brd) family of proteins as key regulators of VSMC phenotype.

In this project we plan to further investigate the role of these BRD family proteins and test for drugs that can 'normalise' the epigenetic signature of VSMCs to treat calcification. We will use human VSMC calcification assays, siRNA knockdown and qRT-PCR and CHIP assays to study gene expression when we perturb BRD protein expression. We will also use confocal microscopy to examine localization of key epigenetic regulators in VSMCs *in vitro* and in human tissue samples *in vivo*. Training in ChIP-seq and bioinformatic analysis of large datasets from ChIP experiments, RNA sequencing and single-cell sequencing datasets can also be provided to validate our findings in large human datasets.

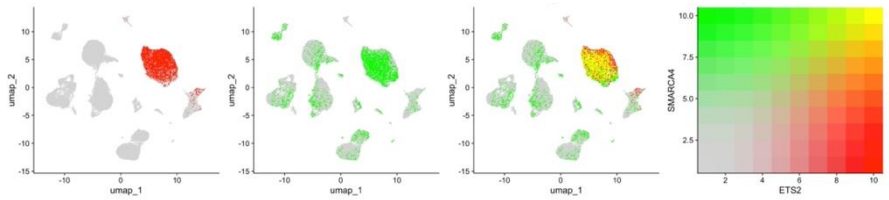


Figure showing changes in BRD epigenetic regulation in a specific subset of modified VSMCs from a sc-sequencing dataset of humanvascular disease.

References:

1. Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM. (2018). Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. *Cardiovasc Res*. 15;114(4):590-600.
2. Liu Y, Drozdov I, Shroff R, Beltran LE, **Shanahan CM**. (2013). Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. *Circ Res*. 10;112(10):e99-109.