KING'S College LONDON

Multiscale Models for Life Centre for Doctoral Training

Project Catalogue

Dear prospective MM4L student,

Welcome! Here is our guidance on choosing a PhD project.

This PhD project is a 3.5-year commitment with major impact on your training and future career. Please read this carefully to ensure that you can make an informed decision about your PhD.

Deciding on Projects and Learning New Things

Here you find the catalogue of PhD projects that you can choose from. Students often want to continue in the same field as their prior training. We strongly recommend that you do something different instead. To decide on a project, consider the learning opportunities that are central to a PhD:

- **Try something new** – such opportunities are rare, and they truly enhance your learning.
- **Broaden** your knowledge and experiences.
- **Maximise learning opportunities.** Don't do more of the same. Move outside your comfort zone. That's how **breakthroughs** happen.
- Be open to **new concepts and experiences** which you may encounter.

You won't know if you'll love it unless you try it! Your experience and education are still relatively narrow compared to the breath of MM4L. Many cutting-edge projects available in MM4L lie outside mainstream topics because of their interdisciplinary nature and originality. Don't reject them out of hand just because they don't lie in your comfort zone.

We recommend that you speak to **at least 3** (and ideally more) co-supervisors about their projects to get a broad sense of what we offer and learn about the breath of interdisciplinary biosciences. Even if you don't select a project, the meeting will be an important educational opportunity where you will learn about a new field from a 1-to-1 meeting with an expert. **You should indicate your 3 project selections on your application form.**

If awarded a studentship: Making Choices About Your PhD and Your Future

Your success in the PhD depends mostly on your environment as well as your working relationship with your supervisors and lab colleagues. **This criterion is far more important than the actual research topic.** (Do not ever pick a project where you love the scientific topic but cannot get along with the supervisor. A passion for skydiving should not override the lack of parachutes.)

You must find out as much about the research and training environment as possible to ensure that you can make an informed decision about where to pursue your PhD.

With over a decade of PhD training experience, the MM4L leadership team recognises that every supervisor-student relationship is unique. A perfect environment for one student might not work at all for another. **It is therefore critical to select a PhD that fits you.** A good fit ensures that you will be trained and supported to bring out your best, whereas a bad fit could compromise your training and affect your wellbeing.

Only accept a project that you are confident will lead to a PhD that fits you. There is **no commitment** to accept a project even if:

- You met with the supervisor(s).

- You indicated a preference at any time.
- You devised the project with the supervisor(s) or the supervisor(s) customised a project for you.

If you do not feel that there is a good fit, it is **totally ok** to decline and choose something else that you feel is a better fit, even if it might be awkward. It is critical to select a PhD that will allow you to flourish.

From our experience with hundreds of PhD students, these are the key factors in the success of a PhD – find out about them:

- The **most important factor** is the supervisor-student relationship.
- Lab/industry environment including people, equipment, and other resources.
- What you will learn (e.g., transferable skills, independence, confidence-building, how to fail and cope with failure).

Meeting with Supervisors and Lab Members

You must schedule meetings with both supervisors **before selecting a project.** The **goal** of these meetings is to set accurate expectations for you and your supervisor. You can thereby determine if the supervisor aligns with your expectations, and vice versa.

During the meeting, you should ask lots of questions, including:

- Get details about the project **again**. Determine precisely what you will do each year and get details of the research plan.
- **How does your project fit in with the rest of the lab?** Will you collaborate with others on one project, or will you have your own niche? How will credit be shared in publications and patents?
- Is the project as **advertised?** Science moves fast; some things can change since the indicative project was advertised.
- Find out about the **lab** and the **people** in it. How many are there and who will be supervising you on a day-to-day basis.
- Are **equipment and reagents in place** for the research?
- **Who will train you? How much time** will they devote to you? Can they train you **from scratch** if you lack certain skills or experience?
- What is the **role of each supervisor?** You will have two supervisors at King's. Depending on the project, you may also have a supervisor from an industry partner. Is it clear how they will work together to supervise your project effectively? **Ask all supervisors.**
- If your project involves **industry collaboration**, you can ask to see the contract agreement with the industry partner to look at resources and in-kind contribution as well as agreements regarding publication, your thesis, confidentiality, and Intellectual Property. You should also ask if there will be an **industry placement**, for how long, when, what will you be doing, and how will it contribute to your PhD training and thesis?

Tell your supervisors the following:

- Explain your **background and ability** so that they understand your training needs. Be sure that they are committed to provide the training that you need.
- Explain your **disabilities or conditions**. Ask them how they will accommodate you. The offer cannot be withdrawn because you have a disability or condition.
- Institutions (and your supervisors) have a legal requirement and moral responsibility to make reasonable accommodations to every student. There are many resources including additional funding for this purpose. But accommodations can only be made if you disclose your disability or condition.

You should also meet with students and/or postdocs from the lab, **without the supervisor present**. Ask about:

- **The supervisor's personality and working style.** How do they interact with and supervise people in the lab?
- **Lab culture.** Is the lab very social or very business-like? Do people tend to work individually or collaboratively? How much help and guidance can you expect from others in the lab?
- If you will be trained by people other than the supervisor, find out **how much training and time** they will devote to you.
- **Verify** everything the supervisor told you about the availability of reagents and equipment, your role in the project, and how credit will be shared.

If logistically feasible, also consider visiting the lab to see the actual space.

Equality, Diversity, and Inclusion (EDI)

MM4L is highly committed to EDI during recruitment and supporting our PhD students. We also ask students to consider the multifaceted aspects of EDI when deciding on a PhD project.

- Supervisors have **diverse** traits – they belong to different ethnicities, genders, sexual orientations, religions, ages, and may have disabilities or currently be pregnant / on maternity leave.
- Be aware of your **unconscious bias** and take that into consideration when selecting projects and supervisors.
- Be **inclusive** – make reasonable accommodations for any traits or conditions supervisors might have.

Great PhD projects can be found in all sorts of labs. The extra effort to look at a broad range of labs will pay off.

Questions?

For additional information, please contact Dr QueeLim Ch'ng (queelim@kcl.ac.uk) or Prof Karen Liu (karen.liu@kcl.ac.uk). Academics on the MM4L team will be happy to address any questions and provide personalised guidance.

Project Reference: MM4L1

Modelling biomechanical contributions to chemotherapy resistance

Co-Supervisor 1: Maddy Parsons, Randall Cell & Molecular Biophysics

Field of Expertise: In Vitro Modelling; Molecular Biology; Translational Medicine

Co-Supervisor 2: Rachel Bearon, Mathematics

Field of Expertise: Computational Modelling; Bioinformatics; Translational Medicine

Contact: maddy.parsons@kcl.ac.uk, rachel.bearon@kcl.ac.uk

Objectives

Breast cancer is the most common form of cancer in the UK, with ~55,000 diagnoses and 11,500 deaths from this disease per year. Whilst >90% of patients diagnosed with early disease survive more than 5-years, many patients with more advanced disease or specific cancer subclasses do not respond to therapy. This is because these tumours are complex and different in each patient, and because we have a poor understanding of the biology. Our recent data indicates that the biomechanical properties of breast cancer in patients directly correlates with response to chemotherapy. However, how tumour cells from different patients alter their mechanical stiffness in response to chemotherapy, and how this alters efficient chemotherapy uptake remains unclear. This project aims to combine patient-derived organoid models, advanced imaging, computational analysis, and mathematical modelling to understand how altered biomechanical properties of tumours and complexities of contributions from other cell types affects chemotherapy delivery and cancer cell survival. Data arising from this project will provide insight into contributions from the tumour microenvironment to therapeutic response and provide novel drug discovery approaches and model for treatment of cancer patients.

Workplan

The project will comprise wet lab experiments, computational analysis and advanced mathematical modelling to develop a more holistic understanding of how the biomechanical properties of the tumour microenvironment affect breast cancer cell response to treatment. The project will comprise 3 overarching aims:

1. Multimodal imaging of drug uptake and functional response in breast cancer organoids. The student will learn how to culture human patient-derived breast cancer organoids and embed in collagen gels of increasing stiffness to recapitulate the tumour microenvironment. Organoids will be treated with fluorophore-labelled chemotherapy agents (taxanes, cisplatin) separately or combined and imaged daily using non-invasive fluorescent probes to mark nuclei and cell membranes. Live high-resolution imaging over treatment time courses will be used to detect uptake of drugs and subsequent functional responses. Orthogonal imaging approaches such as spatial mass spectrometry will be applied to detect spatial drug uptake/proteome changes at fixed time points and correlate with function. Computational image analysis pipelines will be developed to correlate drug uptake/exposure with spatial responses depending on biomechanical properties of ECM.

2. Computational modelling of drug uptake in response to biomechanical environment. The students will develop a computational workflow to capture the spatial-temporal uptake of drugs within organoids and cancer cell responses (proliferation, invasion etc). This framework will be parameterised and tested against data from the above experimental pipelines, and the impact on

model parameters of the biomechanical properties of the ECM will be rigorously quantified through in silico simulations, the computational framework will provide predictions for more complex settings (e.g., co-culture), and enable the iterative testing of emerging concepts to inform experimental design.

3. Define stromal cell contributions to drug response.

To expand on the tumour-only models, experimental data will be gathered to incorporate inclusion of stromal cells (fibroblasts, immune cells) and imaging conducted to understand how these cells influence responses to biomechanical properties of the matrix and ensuing functional ednpoints. Resulting data will be incorporated into models to provide more complex frameworks to understand multi-cell interactions and predictive modelling of changes to cell density, stiffness and functional outcomes in response to standard of care therapies. Models will be further refined through incorporation of clinical metadata to determine contributions from any genetic mutations or other clinical features to explore heterogeneity in cell response to treatment.

The outputs arising from this project will provide robust experimental and computational pipelines to understand complex interactions within the tumour microenvironment and contributions from biomechanical and cell-specific interactions in therapeutic response. We anticipate this will be broadly applicable to other tumour types and provide open access computational models for broader testing of drug response in both academic and industry drug discovery settings. Importantly, the project will equip the student with excellent, highly desirable skill sets at the interface between advanced experimental and data/computational domains, enabling them to be fluent in both biological and computational languages.

Project Reference: MM4L2

Experimental and computational analysis of neuroendocrine networks that link diet to ageing at single-cell level

Co-Supervisor 1:	QueeLim Ch'ng, Developmental Neurobiology
Field of Expertise:	Animal Modelling; Bioinformatics; Molecular Biology; Computational Modelling; Development
Co-Supervisor 1:	Sophia Tsoka, Informatics
Field of Expertise:	Bioinformatics
Contact:	queelim@kcl.ac.uk, sophia.tsoka@kcl.ac.uk

Objectives

How neural networks perform sophisticated computations is a fundamental question. In all animals, brain-body communication plays a central role in modulating ageing in response to diet. This project combines neuroscience, genetics, and physiology to address how a C. elegans neuroendocrine network in the nervous system encodes diet to modulate lifespan. We have previously shown that this network comprises gene regulation among neuroendocrine signals emanating from distinct neurons. These neuroendocrine signals are highly conserved, enabling studies in the experimentally tractable roundworm C. elegans to provide new insights into the neuroendocrine regulation of ageing.

Workplan

This project will uncover the pattern of connections among food-encoding genes and neurons, thereby revealing how this network performs novel forms of neural computations during processing of nutrient information.

The student will use cutting-edge techniques in single-neuron transcriptomics, microscopy, molecular genetics, data science, machine learning / artificial intelligence, and network analysis. This project will expose the student to concepts in system biology that relate network connectivity to computation. This project is organised around three aims:

1. Delineate network connectivity by using single-neuron transcriptomics to uncover co-expression gene modules in every neuron among mutants and across different dietary conditions.

2. Develop an integrated experimental and computational pipeline for single-molecule fluorescence in situ hybridisation (HCR3.0) to measure gene expression levels and validate the results from single-neuron transcriptomics analysis.

3. Build models of the network based on experimental data and identify computations mediated by specific connections.

These aims roughly correspond to years 1-3 of the PhD, with the final 6 months for tying up loose ends and writing the thesis. Training and research activities are detailed below.

In Year 1, the student will perform single-neuron RNA-sequencing to profile the neuronal transcriptomes and to reveal gene network activity patterns that encode food stimuli in wild-type and mutants in food sensing pathways, such as serotonin, TGF-beta, and insulin. They will be be exposed to C. elegans genetics and transcriptomics as well as coding, machine learning, and

bioinformatics to analyse the transcriptomics data. They will also initiate training in image analysis in preparation for Year 2. During the first year, students will participate in MM4L training activities.

In Year 2, the student will perform HCR3.0 to validate the single-neuron transcriptomics results and analyse the data. These research activities will build on the student's Year 1 training. The student will also prepare for the upgrade that will take place by 18 months from the start of the PhD. They will also determine the impact of genetic mutations on food encoding across the nervous system.

In Year 3, the student will analyse the neuroendocrine network to uncover its computations and emergent properties. They will perform network inference based on wild-type and mutant single-cell transcriptomes from both internal and public sources. Next, they will analyse the networks with machine learning approaches to identify network properties. Combining these results with the data from Year 1-2 will reveal how gene regulatory connections mediate computations.

In Year 4, the student will complete all research and start writing up the PhD thesis. The last 3 months will be devoted to full-time writing.

Project Reference: MM4L3

Intestinal exosomes as key transporters across the intestinal barrier

Co-Supervisor 1: Joana F Neves, Centre for Host Microbiome Interactions

Field of Expertise: Stem Cell Biology; Translational Medicine

Co-Supervisor 2: Driton Vllasaliu, Institute of Pharmaceutical Science

Field of Expertise: In Vitro Modelling; Molecular Biology

Contact: joana.pereira_das_neves@kcl.ac.uk, driton.vllasaliu@kcl.ac.uk

Objectives

Transport of nutrients, bacterial products, allergens and drugs across the intestinal barrier is key for a healthy gut and its dysregulation can lead to local and systemic diseases. The key players in this process are still being elucidated. A possible candidate are exosomes, which are membrane-bound extracellular nanovesicles secreted by most cells. Much attention has been recently devoted to exosomes as they play an important role as mediators of intercellular communication, having a distinct ability to carry small molecules, proteins, and nucleic acids to recipient cells. However, their role on intestinal pathophysiology, via intestinal mucosa cell-cell communication is unknown.

This project will build complex organoid models and use interdisciplinary approaches to characterise intestinal epithelial derived exosomes and determine how they modulate epithelia and immune cell function, which are key for intestinal health.

Objective 1: To develop an in vitro model of human intestinal organoid monolayers co-cultured with lymphocytes.

Objective 2: To characterize exosomes produced by intestinal epithelial cells in physiological conditions and after challenge with bacterial metabolites.

Objective 3: To determine how exosomes impact epithelial and lymphocyte function in vitro and in vivo in physiological conditions and in the context of intestinal inflammation.

Workplan

Work Package 1. Establishment of a human intestinal organoid monolayer-immune cell co-culture system.

Human intestinal organoids will be cultured on Transwell filters following methodology which Vllasaliu has successfully developed recently (Zhang et al. Molecular Therapy Nucleic Acids. In Review). Cells will be imaged daily (light microscopy) to monitor growth and transepithelial electrical resistance (TEER) will be used to indicate tight junction formation. The monolayers allow for easy access to the basal (immune cells site) and apical (bacteria/metabolites/drugs site) of the intestinal epithelial cells permitting easy manipulation of these cultures.

Blood or intestinal harvested lymphocytes, such as Innate Lymphoid Cells, will be added to the basal side of the epithelial cells. The Neves lab has successfully established 3D cultures of intestinal organoids with immune cells (Jowett et al, Nature Materials 2021 and Cell Reports 2022) and will use this knowledge to develop the 2D organoid monolayer immune co-culture model. The growth and phenotype of cells in co-culture will be monitored by imaging and TEER measurement.

At the end point, transmission electron microscopy (TEM) will be employed to reveal the overall morphology, including the presence of microvilli. Then phenotype of epithelial and immune cells will be determined by several methods including, immunocytochemistry, flow cytometry and RT-PCR.

Work Package 2. Characterization of intestinal epithelial cell-derived exosomes.

Exosomes are produced by all cell types and our preliminary data confirms their production by human intestinal organoids. The supernatant of the organoid monolayer cultures will be harvested and exosomes isolated using established ultracentrifugation-based approaches. Exosomes will be characterised using state-of-the-art nanotechnology and analytical tools, including for size, number, surface charge, the expression of key exosomal protein markers and protein composition via proteomics. These measurements will be done at steady and after challenge of the organoid monolayers with bacterial metabolites (such as butyrate).

Work Package 3. Determination of the impact of intestinal exosomes on epithelial and immune cells.

Exosomes produced by monolayer organoids and metabolite challenged organoids will be isolated as above and added to the immune-organoid monolayer cultures. The impact of these exosomes on epithelial and lymphocyte phenotype will be determined as described in work package 1.

To test these effects in vivo, fluorescently labelled exosomes will be administered into wild-type mice by oral gavage, and their biodistribution, as well as impact on intestinal barrier function and immune and epithelial cell phenotype will be determined at steady-state and in intestinal inflammation (DSS-induced colitis).

Exosomes are a great candidate to delivery new therapies. Thus, its crucial to understand their physiological role and how they are modulated by the intestinal environment such as the microbiota (and their metabolites) and any impact they might have on crucial intestinal players such as the epithelial cells and the intestinal lymphocytes.

Overall, this project will build a new intestinal model that will allow to dissect the role of intestinal exosomes and by using a combination of in vitro and in vivo systems it will explore exosome functions in the gut in health and disease.

Project Reference: MM4L4

A dynamic map of intra-mitochondrial viscosity and diffusiveness in healthy, diseased, and aged neurons

Co-Supervisor 1:	Alessio Vagnoni, Basic & Clinical Neuroscience
Field of Expertise:	Animal Modelling; In Vitro Modelling; Molecular Biology; Live cell imaging, Optogenetics
Co-Supervisor 2:	Simon Ameer-Beg, Comprehensive Cancer Centre
Field of Expertise:	Physics, engineering, optics, bioimaging and biophysics
Contact:	alessio.vagnoni@kcl.ac.uk, simon.ameer-beg@kcl.ac.uk

Objectives

1. To measure the viscosity of the mitochondria in different neuronal compartments (for example, cell bodies, axons, synaptic terminals) ex vivo in Drosophila. This will generate the first map of mitochondrial viscosity within living neurons in an organismal context.

2. To characterise mitochondrial viscosity as they are transported within the axons of neurons. This will show whether different populations of mitochondria, for example stationary vs motile, are characterised by a specific viscosity profile.

3. To test whether mitochondria under tension, usually observed during mitochondrial dynamic events, adjust their microviscosity profile. We will specifically model a pre-mitochondrial division state by inducing mitochondrial stretching through optical trapping as well as using appropriate Drosophila genetic backgrounds.

4. To analyse intra-mitochondrial protein diffusion and correlate the extent of mitochondrial content mixing with the viscosity state of the organelle. Diffusion will be at first measured through micro-rheology experiments by engineering a new genetically encoded mitochondrial probe.

5. To test whether viscosity of different mitochondrial population is affected in specific genetic backgrounds modelling neurodegeneration and accelerated ageing.

Workplan

1. The student will first learn how to culture the fruit fly Drosophila melanogaster and to obtain a ‘larval fillet’ preparation amenable to high-resolution live microscopy. Drosophila motor neurons will be stained through bathing in a solution of the molecular rotor FMR-1 and imaged ex vivo on a Leica Stellaris Dive FALCON commercial system in the Centre of Excellence for Fluorescence Lifetime Imaging within the Microscopy Innovation Centre as well as a bespoke Massively Parallel Fluorescence Lifetime Imaging (mpFLIM) microscope in the Ameer-Beg lab.

Mitochondrial viscosity will be measured by FLIM to exploit the direct correlation between viscosity and lifetime of the FMR-1 dye. Because mitochondrial morphology and function are known to be partly dependent on the subcellular location of the organelle, the student will focus on understanding if mitochondrial viscosity is compartment dependent. They will measure the viscosity in the cell soma, at the synaptic terminals and in the proximal, mid, and distal portions of the axons. This initial characterisation will provide a novel map of neuronal mitochondrial viscosity and function as essential benchmark for future studies.

2. The student will move onto more detailed analyses by characterising the viscosity of moving and static mitochondria in the axons of neurons in real time. Because mitochondria transported in opposite directions (towards the cell body and synaptic terminals, respectively) are thought to be functionally distinct, this viscosity analysis will be further stratified by studying mitochondria that move in an anterograde and retrograde direction within axons.

3. We hypothesise that the dynamic state of mitochondria correlates with a specific viscosity profile. To directly test this hypothesis, the student will use Drosophila genetic backgrounds in which fission and fusion proteins are depleted to trigger elongated or shortened mitochondria, respectively, and measure their viscosity. The student will also directly be able to apply force on the mitochondria to induce membrane stretch and measure the viscosity in this context. This innovative approach will be achieved by using a force calibrated optical tweezer system (Impetux SensoCell), unique in the UK, integrated in a SWARM FLIM system within the Ameer-Beg lab.

4. Molecular diffusion is correlated to viscosity by the Stoke-Einstein equation. The student will perform micro-rheology experiments by developing a new genetically-encoded mitochondrial targeted nanoparticle probe (mito-GEM) to characterise protein diffusion in the matrix of the mitochondria located in different neuronal subcellular locations. This will be based on our previous work with the cytoplasmic GEM sensor (https://www.biorxiv.org/content/10.1101/2023.09.18.558344v1) coupled to single particle tracking to measure cytoplasmic diffusiveness. If we are not able to track the GEM particles in the constrained space of the mitochondria, we will use FRET analysis of a soluble GFP reporter.

5. The student will be able to test whether Drosophila strains expressing human proteins causing ALS, Parkinson disease and Alzheimer's disease display compartment-dependent and strain-specific mitochondria microviscosity profiles. Fly neurons overexpressing progerin will be used to model accelerated ageing. If time allows, these studies can be expanded into an in vitro human model of neuronal ageing already in use in the Vagnoni lab.

Project Reference: MM4L5

Building physics-informed Neural Networks to bridge the gap from micro- to macro- modelling of human cortical folding development

Co-Supervisor 1: Katie Long, Developmental Neurobiology

Field of Expertise: In Vitro Modelling; Development; Stem Cell Biology; Biomaterials

Co-Supervisor 2: Emma Robinson, Biomedical Engineering

Field of Expertise: Computational Modelling

Contact: katie.long@kcl.ac.uk, emma.robinson@kcl.ac.uk

Objectives

The shape of the human brain has long been linked to its function, with changes in shape often associated with changes in cognition. Subtle changes in shape, often called malformations of cortical development (MCDs), can occur in many neurodevelopmental disorders, suggesting regulation of brain shape is an important developmental process. MCDs are thought to be associated with disruptions to the processes of cell proliferation and apoptosis, cell migration, and post-migrational development.

However, how the human brain develops to the right shape, and what goes awry in MCDs is not fully understood. There has been an understanding that the process of cortical folding results from biomechanical tensions as the cortex expands. However, a complete model of the process remains elusive. Plausible theories include more rapid growth of the cortex relative to sub-cortical white matter [Tallinen 2014], and the impact of axonal tension as neuronal connections form [Van Essen 2020]. But these are incomplete and can only partially replicate the patterns of cortical folding observed in live human brains.

This project combines cellular and Physics-informed neural network (PiNN) simulations to bridge the gap between in vitro/ex vivo and in silico tissue models of cortical folding to macroscale observations from whole brain imaging.

Workplan

Overall, the objective is to build models that incorporate biomechanical constraints, using PiNNs to make predictions that can be tested in the tissue/cell-based model systems. The cellular models will use induced pluripotent stem cells (iPSCs) from individuals with typical and atypical brain shapes, grafting of these cells into human cortex explant cultures. The fetal tissue that the Long lab works with is during the critical window of fetal brain development (12-20 pcw) where many of the cell behaviours listed above occur (proliferation, migration and apoptosis), providing a unique model to investigate changes in cell behaviours within a physiological tissue environment. Imaging and analysis of the differences in cellular processes will be combined with computational modelling to understand how altered cell behaviours in development lead to changes in brain shape.

Machine-learning will be used to optimise biomechanical models of cortical growth and deformation [Da Silva 2021] from simulated data that will learn to mimic the behaviour of the tissue cell models. In this way it will be possible to train an AI to design entirely new models of tissue folding that much more closely reflect the in vivo patterns of cortical folding that we observe from longitudinal fetal MRI data. The overarching objective will be to see if we can simulate (in silico and in vitro) the development of cortical malformations such as polymicrogyria and lissencephaly.

This information will be used to inform the wet lab side of the project, which will build tissue models (Long et al, 2018) in which these biomechanical parameters can be tested. For example, would altering tissue stiffness result in abnormal folding of the cortex? We can also combine the tissue with neural cells derived from patient iPSC lines. These will be selected from patients with known structural malformations of the cortex, or can alternatively be manipulated to express mutations known to result in cortical folding malformations. The biomechanics of these cells in the tissue environment, for example their migration speed and direction, could then be used to inform the in silico models to investigate if this would lead to the structural change seen in the patient MRI.

Tallinen, Tuomas, Jun Young Chung, John S. Biggins, and L. Mahadevan. 2014. “Gyrification from Constrained Cortical Expansion.” Proceedings of the National Academy of Sciences of the United States of America 111 (35): 12667–72. doi:10.1073/pnas.1406015111.

Van Essen, David C. 2020. “A 2020 View of Tension-Based Cortical Morphogenesis.” Proceedings of the National Academy of Sciences of the United States of America 117 (52). Proceedings of the National Academy of Sciences: 32868–79. doi:10.1073/pnas.2016830117.

Long KR, Newland B, Florio M, Kalebic N, Langen B, Kolterer A, Wimberger P, Huttner WB (2018) Extracellular matrix components HAPLN1, lumican and collagen I cause hyaluronic acid-dependent folding of the developing human neocortex. Neuron 99: 702-19.

Silva, Mariana Da, Carole H. Sudre, Kara Garcia, Cher Bass, M. Jorge Cardoso, and Emma C. Robinson. 2021. “Distinguishing Healthy Ageing from Dementia: A Biomechanical Simulation of Brain Atrophy Using Deep Networks.” In Machine Learning in Clinical Neuroimaging, 13–22. Springer International Publishing. doi:10.1007/978-3-030-87586-2_2.

Project Reference: MM4L6

Tissue-scale mechanics in pancreatic cell fate decision and shape acquisition

Co-Supervisor 1:	Francesca M Spagnoli, Gene Therapy and Regenerative Medicine
Field of Expertise:	Animal Modelling; In Vitro Modelling; Development; Stem Cell Biology; Translational Medicine
Co-Supervisor 2:	Sergi Garcia-Manyes, Physics
Field of Expertise:	Physics, Mechanobiology
Contact:	francesca.spagnoli@kcl.ac.uk, sergi.garcia-manyes@kcl.ac.uk

Objectives

Spatiotemporally regulated reciprocal interactions between epithelial cells and surrounding microenviroment(s) are essential to build functional organs but also to maintain adult organ homeostasis. The pancreatic microenvironment is composed of multiple cell types, including mesenchymal cells, endothelial cells, neural and immune cells as well as the ECM. The interplay between pancreatic epithelium and its microenvironment is fundamental for the development of a proper functional pancreas, including cell differentiation and growth. Despite the relevance of such tissue interaction(s), very little is known about the different sets of cellular interactions that occur in the developing pancreas between pancreatic cell types and surrounding cells as well as ECM components. Moreover, it is unknown whether microenvironmental signals establish 'niches' with distinct mechanical properties, which in turn might control different aspects of pancreatic tissue formation. In this project, we aim at understanding how mechanics regulate cell behaviours in the context of pancreatic development and how to use these insights to help guide the strategy and design of functional engineered tissues.

Workplan

The Spagnoli laboratory has longstanding interest in intrinsic and extrinsic mechanisms regulating pancreatic cell identity. They set up ex vivo and in vivo models to study the composition of the pancreatic microenvironment in the mouse embryo and human tissue. They focus in particular on the pancreatic mesenchyme, defined its heterogeneity at the transcriptome level and identified a putative "niche" controlling pancreatic differentiation. The Garcia-Manyes laboratory focuses on mechanobiology across different length-scales. They use a combination of bespoke nanomechanical techniques at the single molecule and at the single cell level to interrogate how mechanical forces affect protein folding and how cells react to mechanical forces.

The proposed multidisciplinary project will build on our previous findings and combine our complementary expertise. We aim at resolving the full spectrum of cellular interactions in pancreatic tissue across space and time and how they provide mechanical cues to aid in controlling cell and tissue structure and function.

Specific goals of the project are:

1) To spatiotemporally resolve mechanical properties across mouse pancreatic embryonic tissue and organ cultures using atomic force microscopy (AFM) combined with fluorescence microscopy.

2) To integrate mechanical properties with microenvironmental features, including cellular interactions, ECM and signaling molecules distribution. To this aim, we will use spatial transcriptomic datasets of mouse pancreatic embryonic tissue recently generated in the Spagnoli lab.

3) To characterize the functional properties of selected putative mechanical “niches” that will be defined in aims 1 and 2 using mouse pancreatic embryonic organ cultures and synthetic iPSC-derived models.

Overall, these studies will shed light onto how tissue-scale mechanics is involved in controlling cell fate acquisition and morphogenesis during organ formation.

Project Reference: MM4L7

Artificial intelligence and bioinformatics approaches to assess predictive biomarkers for immunotherapy and investigate optimum treatment combinations in head and neck squamous cell carcinoma (HNSCC)

Co-Supervisor 1:	Heba Sailem, Institute of Pharmaceutical Science
Field of Expertise:	In Vitro Modelling; Computational Modelling; Bioinformatics; Translational Medicine
Co-Supervisor 2:	Anthony Kong, Comprehensive Cancer Centre
Field of Expertise:	Translational Medicine; clinical trial and molecular oncology; In Vitro Modelling
Contact:	heba.sailem@kcl.ac.uk, anthony.kong@kcl.ac.uk

Objectives

Rationale

The treatment options for head and neck squamous cell carcinoma (HNSCC) include a combination of surgery, radiotherapy or chemotherapy. For patients who present with recurrent/metastatic (R/M) HNSCC, anti-PD1 checkpoint inhibitor is approved as monotherapy but it only benefits a small number of R/M HNSCC patients. Thus, there is an urgent need to find biomarkers that could identify patients that would benefit from these treatments and to find optimum treatment combination that could help to improve survival for these patients.

Aim

The overall aim of this study is to use bioinformatic and machine learning approaches to decipher the tumour microenvironment and to find potential predictive biomarkers and optimum treatment combinations in head and neck squamous cell carcinoma (HNSCC).

Specific research objectives

1. To perform and analyse exome sequencing and RNA sequencing of the primary and recurrent samples from HNSCC patients

2. To assess the tumour immune markers and peripheral immune effect of HNSCC patients treated with radiotherapy, chemotherapy and/or immunotherapy, to identify relevant biomarkers

3. To integrate the tissue image data using Artificial intelligence and bioinformatics approaches to find predictive biomarkers and optimal treatment combinations

4: To validate the predicted optimal treatment combination in patient-derived organoids PDOs and mouse models

Workplan

Objective 1: To perform and analyse exome sequencing and RNA sequencing of the primary and recurrent samples from HNSCC patients

We are currently conducting SOTO study (chief PI: Dr. Anthony Kong, NCT 05400239, new completion date June 2025). In this study, we generate patient-derived organoids (PDOs) from HNSCC patients undergoing surgery, radiotherapy or immunotherapy. We aim to assess whether we can use PDOs to guide patients' treatment. In this PhD, the candiate will analyse the exome-seq and RNA-seq sequencing data from paired primary and recurrent/metastatic HNSCC from the SOTO study. For the primary tumour samples, we will also perform multi-regional sequencing to assess intratumour heterogeneity. We will also correlate the genetic profiles of organoids with that of patients' samples. In addition, the candidate will assess the genetic landscape and the gene expression and immune profile patterns change during tumor evolution and when recurrence/metastasis occur and how these changes may affect response to subsequent immunotherapy treatment.

Objective 2: To assess the tumour immune markers and peripheral immune effect of bloods from HNSCC patients treated with radiotherapy, chemotherapy and/or immunotherapy, to identify relevant biomarkers

In SOTO study, patients will undergo various standard treatments including radiotherapy, chemotherapy and/or immunotherapy. The candidate will develop a detail investigation of total immune profile of the primary and recurrent HNSCC samples of these patients in SOTO study. We will assess the multiplex IHC imaging of the immune markers including CD4 and CD8-T markers, FoxP3, PD1/PDL1, myeloid markers as well as other immune markers. The candidate will also assess peripheral immune profiles from bloods obtained from HNSCC patients before and after treatments.

Objective 3: To integrate the complex data using Artificial intelligence and bioinformatics approaches to find predictive biomarkers and optimum treatment combination

You will integrate data from different biological scales in order to identify predictive biomarkers of patient response to treatment. This will allow identifying how signatures at the molecular scale (RNA seq and exosome sequencing) correlate with the cellular and tissue level information. You will utilise mechanistic modelling approaches that allow testing identified hypothsis. Additionally, deep learning approaches such as graph neural networks will be trained to determine if such models could support clinical decision making for pre-treatment biopsies.

Objective 4: To validate the optimal treatment combination in PDOs and mouse models

In addition, the candidate will learn how to grow PDOs from tumour samples of primary and recurrent HNSCC (+ surrounding normal tissues) and subject them to different treatments including as predicted from obejctive 3. For optimum treatment combination involving immunotherapy, the PDOs will be co-cultured with matched immune cells from SOTO study. In addition, we will assess optimum treatment combination in mouse models including syngeneic HNSCC models (e.g. MOCL1 and MTCQ1 cells) involving immunotherapy.

Expected outcome

This is a truly multidisciplinary project and the candidate will learn both dry (bioinformatics data analysis) and wet experiments (generation of head and neck organoids and biochemical analysis). The candidate will also learn about clinical and translational research as well as reverse translation.

Project Reference: MM4L8

Many ways to grow a bone: an evolutionary development and mechanical simulation investigation into the growth and development of sesamoids bones

Co-Supervisor 1: Michael Berthaume, Engineering

Field of Expertise: Computational Modelling; macroevolutionary modelling

Co-Supervisor 2: Abigail Tucker, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Stem Cell Biology; Development

Contact: michael.berthaume@kcl.ac.uk, abigail.tucker@kcl.ac.uk

Objectives

Sesamoids are variably present bones located in tendons and ligaments, often near joints. They form in embryonic development in response to both molecular and mechanical (i.e., muscular) cues. As they grow, they function as "pulleys", redirecting muscle forces and alleviating stresses in the tendons/ligaments. In humans, the presence/absence of knee sesamoids can influence susceptibility to knee pain, osteoarthritis, and affect knee surgeries.

Recent evolutionary models on primate knees have suggested there is more than one way to grow and evolve sesamoids. For this project, the student will combine developmental biology and evolutionary biomechanics to reconstruct the evolution and formation of knee sesamoids in placental mammals. To do so, the below objectives will be followed:

1) Analyse developmental series of foetal knees of a variety of mammalian species, including mouse and human, using molecular and morphometrics approaches, complemented by data gathered from the mammal collections at the Natural History Museum.

2) Model the growth, development, function, and evolution of sesamoids in the knees of mice and humans using biomechanical, finite element, musculoskeletal, and statistical models. Models will be validated using morphometric data.

Workplan

The following workplans (WPs) will be conducted:

WP0 Literature review (months 0-3): For the first three months, the student will conduct a literature review. Papers will include existing hypotheses concerning sesamoid growth, development, and evolution in mammals, bone growth, development, and biology, and biomechanical modelling of bone.

WP1 Development (months 3-15): Embryonic and foetal knees will be analysed by microCT and using thin sections to visualise development of knee sesamoids with a focus on mouse and human tissue (provided by the Human Developmental Biology Resource). The mouse data will be compared to knees from CACNAS1 mutant embryos, which have a defect in muscle contraction, to understand how force impacts sesamoid formation. In these mice some sesamoids fail to form while others appear to initiate as normal, highlighting the complex nature of the mechanical and chemical signalling environment. Tissue will be analysed for changes in gene and protein expression.

WP2 Deep time evolutionary modelling (months 6-24): A systematic review of sesamoid presence/absence in mammals will be produced, combining published data and collections at the

Natural History Museum. An updated phylogeny will be created. Phylogenetic comparative methods will be used to investigate the tempo and mode of evolution of knee sesamoids in mammals, and to investigate if sesamoid presence/absence is correlated with any ecological parameters, such as mode of locomotion.

WP3 Evolutionary biomechanics (months 12-36): Two sets of simulations will be run. First, thin sections from WP1 will be used to construct 2D finite element (FE) models of knees. Muscle forces, estimated using muscle cross-sectional area will be applied to the model to simulate muscles contracting in utero, and iterative simulations will be used to model sesamoid growth. Two model geometries will be used. First, where sesamoid ossification centres are attached to primary bones (e.g., as is the case with the patella and femur). Sesamoid detachment and creation of the synovial joint will be simulated. Second, tendons without sesamoids will be modelled to investigate if strains necessary for sesamoid formation occur independently in the tendon.

In the second set of simulations, species of interest identified from WP2 will be identified and chosen for musculoskeletal modelling. Using geometrical data derived from microCT scans, changes in lever arm mechanics of the muscle through knee flexion will be examined.

This project is feasible for a PhD studentship as background work has already been completed, including applying for permissions to access relevant foetal knee collections and a systematic review of sesamoids in placental mammal knees (conducted in 2020, to be updated during project).

The research will shed light on the functional role of sesamoids in mammals, and explain how differences in knee sesamoid anatomy occur in humans. Such knowledge can impact our understanding of human knee health, with the potential to develop therapeutics and physiotherapies that can alleviate knee problems, such as osteoarthritis, by incorporating an understanding of sesamoid anatomy.

Project Reference: MM4L9

Modelling Oral Lubrication Mechanisms: Bridging Simulation and Sensation

Co-Supervisor 1: Sorin-Cristian Vlădescu Engineering

Field of Expertise: Computational Modelling; Development;Biomaterials

Co-Supervisor 2: Guy Carpenter, Centre for Host Microbiome Interactions

Field of Expertise: Biomaterials

Contact: sorin-cristian.vladescu@kcl.ac.uk, guy.carpenter@kcl.ac.uk

Objectives

Meat and dairy alternatives are humanity's top lever to fight climate change, reducing greenhouse gases 11 times more efficiently per investment dollar than zero-emission cars. A major biological barrier to the widespread adoption of plant-based diets is astringency, a loss of oral lubrication while consuming plant-based proteins, leading to lower palatability.

Our current research into plant-based astringency reveals that particles within protein preparations can have a large (30%) effect in causing delubrication of the salivary film on the tongue, leading to the perception of dryness. Our previous work on carbonated beverages also revealed bubbles can affect taste and mouthfeel by affecting the salivary film on the tongue. In this project we believe the properties of the particles and their interaction with the salivary film on the tongue can be harnessed to create novel fat replacement additives. It is known that fat affects mouthfeel and is used in food products to reduce astringency, given the strong correlation between fat percentage and measured friction.

This project brings together a multi-disciplinary team to analyse particle movement under shear conditions to create models which can predict lubrication profiles for in-vivo testing. The aim is to characterise the mechanisms through which particles influence sensory response.

Workplan

The aim of the proposed experimental work is to determine the mechanisms affecting oral lubrication by foods. Specifically, to understand the astringency (reduced oral lubrication) or creaminess (increased oral lubrication) behaviour of different particles, we will measure how the particles behave in vitro, then model mechanical forces induced by particles and finally test different particles in vivo to determine how they are perceived inside the mouth and how humans sense movement at mucosal surface level. We will study how fat molecules behave inside the oral cavity and how their mechanical behaviour can impact the perception of various food products. The project aims to achieve the following deliverables:

WP1: Direct observation of the flow and deformation inside the oral contact of the plant proteins and fats. We will employ a unique, newly designed experimental setup mimicking the physical and mechanical characteristics of the human tongue. Its components include: (a) 3D-printed biomimetic tongue (already developed at King's College), (b) custom-designed oral tribometer simulating complex, real-life tongue movements, (c) optical microscopy combined with fluorescence detection of salivary and food proteins by prior labelling. Particles to be studied will start with fat liposomes, then compare them to polystyrene, silica or mica particles (with different charges) and then plant proteins.

WP2: Computational modelling in the context of taste and oral tribology. FEA (Finite Element Analysis) will be employed for the first time as a tool to study tissue deformation inside the oral cavity. We will begin by creating a digital representation of the oral tissues (i.e., the tongue and the palate), discretising the geometry into interconnected elements to approximate the continuous behaviour of the oral tissue. The FEA model will provide detailed information about the stress, strain, and deformation patterns within the oral tissues. This information is crucial for understanding how different particles entrapped inside the oral contact (information collected under WP1) impact the tissue properties and geometries, and how external forces influence the mechanical response at mucosal level. This will help understand how tissue properties affect food breakdown and bolus formation, ultimately impacting taste perception.

WP3: Integration with Computational Fluid Dynamics (CFD). The FEA model of the oral tissues will be integrated with fluid dynamics simulations for saliva flow and mass transfer models for flavour release, to create a comprehensive understanding of the entire oral processing system of various particles. This will shed light on how cells perceive movement at mucosal surfaces under real life scenarios, when mucins bind to oral surfaces.

WP4: Determining correlations between computational models and microscopy observations, and taste/ sensory assessments from a human panel. Currently, food product development is primarily informed by humans evaluating the taste, texture, mouthfeel etc. of proposed formulations. However, this approach is time-consuming, expensive, and dependent on subjective reporting. Determining correlations between computational models (informed by scientific measurements) and taste perception would allow food manufacturers to decrease reliance on human testers, lowering costs and increasing speed to market by employing a lab-first approach.

Project Reference: MM4L10

Establishing a model of perfused microvasculature

Co-Supervisor 1:	Anna Zampetaki, School of Cardiovascular and Metabolic Medicine & Sciences
Field of Expertise:	Molecular Biology; Stem Cell Biology; Translational Medicine
Co-Supervisor 2:	Pablo Lamata, Biomedical Engineering
Field of Expertise:	Computational Modelling; Bioinformatics
Contact:	anna.zampetaki@kcl.ac.uk, pablo.lamata@kcl.ac.uk

Objectives

Human organoids have emerged as a promising platform to bridge preclinical research with clinical trials. Employing human blood vessel organoids (BVOs) as a model of human microvasculature, we have previously shown that metabolic perturbations can initiate a mechanotransduction cascade and trigger structural remodelling. Blood flow is an important determinant of vascular structure in vivo, but the interplay between flow, metabolism and mechanotransduction in the microvasculature is not well understood.

Objective 1. Establish a microfluidic platform for BVOs.

We will establish a microfluidic system to culture BVOs under flow conditions. BVOs will be exposed to laminar flow and the impact of vascular perfusion on the vascular structure will be assessed. Further experiments targeting PFKFB3, an activator of glycolysis, will elucidate the role of flow in the metabolic reprogramming of the microvasculature.

Objective 2. Generate a 'digital twin' model of the microvasculature.

Computational analysis integrating the vascular features under flow and static conditions with functional information from the key metabolic and proteomic hubs will be used to generate a 'digital twin', i.e. a model of the microvasculature. Computational simulations will then be used to interpret experimental findings, to investigate the key triggers of microvascular dysfunction, and to generate novel hypotheses about the underlying mechanisms.

Workplan

Objective 1. Establish a microfluidic platform for BVOs.

BVOs will be generated using the Wimmer et al protocol. To determine the effect of laminar flow on the microvasculature, BVOs on day 17 of differentiation will be plated on gelbrin hydrogels in microfluidic-based perfusion bioreactors. The gelatin-fibrin substrate that can support strong attachment of organoids under flow conditions will enable a single BVO to be plated in each well in a µ-Slide Spheroid Perfusion Uncoated bioreactor (ibidi). Gelbrin gels will be used to coat the niche area of the wells. For these experiments BVOs with diameter 500µm ± 50 will be used. A total of 21 BVOs in organoid media will be plated in each bioreactor and allowed to attach overnight. The following day, flow will be applied for 5-7 days. Different flow rates, ranging from (200-600mBars) will be used. Flow through the vasculature will be visualised using 40 kDa fluorescein isothiocyanate (FITC)-labeled dextran beads. Functional human microvasculature will be assessed by hCD31, PDGFRβ and FITC labelling. To evaluate the BVO perfusion in response to metabolic stress, BVOs will

be incubated with EBM2 for 24h presence or absence of PFK15 (2.5µM) and/ or CTGF (50ng/ml). Treatment with TNFα (10ng/ml) will be used as a positive control for increased vascular permeability.

Milestone: Establish a perfusion bioreactor for BVOs.

Outcome: Obtain functional readouts for the BVO permeability.

Objective 2. Generate a 'digital twin' of the microvasculature.

Our aim is to generate an in-silico representation of the microvasculature using the structural and functional readouts obtained from BVOs. This model will incorporate features associated with several aspects of vascular rarefaction such as lower vessel density, shorter vessel length, centrality, reduced interconnectivity, incomplete loops, broken branches, decreased pericyte coverage, but also vascular landmarks- vascular end and vascular bifurcations. This will enable us to construct a collection of topological 'vascular network' models. We will then enrich the model with function, and based on the capillary flow we will test the hypothesis that 'poor structural vascular network' leads to functional impairment. The model will also be used to investigate the mechanistic link between metabolic rewiring and structural and functional alterations.

Image J and Matlab software programmes will be used to quantify the vascular network features mentioned above. In the second stage, the functional data obtained in objective 1, using the bioreactor for BVO perfusion and flow patterns at baseline and following stimulation, will be incorporated to the model to produce a simplified version of a 'digital twin' that could be used to identify microvascular dysfunction and predict microvascular perfusion.

Milestone: Establish a computational model of vascular rarefaction

Outcome: Create a computational tool to interrogate the link between structural remodelling in the microvasculature and impaired flow.

Project Reference: MM4L11

Building a multiscale model of cardiac fibrosis and treatment response

Co-Supervisor 1: Alkystis Phinikaridou, Biomedical Engineering

Field of Expertise: Animal Modelling; Translational Medicine; Medical Imaging

Co-Supervisor 2: Gilbert Fruhwirth, Comprehensive Cancer Centre

Field of Expertise: In Vitro Modelling; Molecular Biology; cell tracking; Animal Modelling

Contact: alkystis.1.phinikaridou@kcl.ac.uk, gilbert.fruhwirth@kcl.ac.uk

Objectives

Scientific basis:

Heart failure (HF) affects 64 million people globally and causes sudden death. Cardiac fibrosis (CF) drives HF creating a need to model CF and therapeutically target it. There is currently a lack of a model to study cardiac fibrosis and the effects of novel therapeutics. Thus, building an imaging data-driven model of cardiac fibrosis development and aetiology and how can it be impacted with novel cell-based therapies as anti-fibrotic treatment is needed.

Current imaging technologies do not directly measure CF and no specific antifibrotic drugs are available. Secreted matricellular proteins have been shown to inhibit CF and preserve cardiac contractility in vivo by inhibiting TGFβ signalling. An engineered effector T cell immunotherapy targeting myofibroblasts was reported to treat cardiac fibrosis in mice. Moreover, regulatory T cell (Treg) therapy has been proposed as a candidate to counteract cardiac inflammation- induced CF.

In this project we aim to develop advanced models to diagnose CF and quantify the anti-fibrotic effect of cell-based therapies. To achieve this aim this project will employ a multiscale approach (from cells, to tissue to organ) to directly model the cardiac fibrosis in vivo (Phinikaridou-lab) and use a novel Treg-based therapy to deliver an anti-fibrotic protein (Fruhwirth-lab).

Workplan

Specific objectives for each year:

(1) Build the in vivo animal model of CF and use fibrosis-specific MRI-probes to quantify the extent of cardiac fibrosis (Year 1-1.5).

(2) Build an imaging-based data model of cardiac fibrosis aetiology and progression (Year 1.5-2.5).

(3) Establish an in vivo traceable Treg-cell therapy (by PET imaging) to deliver a known anti-fibrotic protein (AF-Tregs) (Year 2.0-2.5).

(4) Interfere with cardiac fibrosis by administering anti-fibrotic agents including AF-Tregs in vivo and use the cardiac fibrosis model (from Aim 1) to predict therapy efficacy (Year 2.5 to 3.5)

Techniques

- Mouse husbandry, handling, and surgical techniques.
- In vivo PET for cell tracking and MRI to measure cardiac function and fibrosis.

- Tregs cell isolation, purification, and culture
- Build a primary component analysis (PCA) model of the evolution of cardiac fibrosis at a voxel-by-voxel bases using MRI images and treatment response.

Project Reference: MM4L12

Host environmental impacts on bacterial methylation profiles in the human gut microbiota

Co-Supervisor 1:	Jordana Bell, Twin Research & Genetic Epidemiology
Field of Expertise:	Computational Modelling; Bioinformatics
Co-Supervisor 2:	David Moyes, Centre for Host Microbiome Interactions
Field of Expertise:	Animal Modelling; In Vitro Modelling; Bioinformatics; Molecular Biology; Translational Medicine; Microbiology, Immunology
Contact:	jordana.bell@kcl.ac.uk, david.moyes@kcl.ac.uk

Objectives

As part of a recently awarded ERC/EPSRC funded grant we are undertaking novel characterization of bacterial DNA methylation in the adult human gut microbiota, to explore its regulatory potential for bacterial gene function, and its relevance to human diet and health.

The proposed PhD project will integrate within the existing framework to tackle the following specific objectives:

1/ Apply and compare multiple analytical pipelines to quantify bacterial DNA methylation profiles of candidate bacterial species in the human gut microbiome, based on long-read DNA sequencing data.

2/ Characterise variability in the bacterial DNA methylation of candidate species in human cohorts.

3/ Explore if host environmental factors, such as medication use, leave a signature on the gut bacterial methylome.

4/ Test whether particular bacterial DNA methylation signals are associated with microbially derived metabolite levels.

Outcomes. This project will characterise human gut bacterial DNA methylation as a novel tool to uncover molecular pathways mediating microbiome impacts on human health. The results have potential to uncover novel mechanisms of bacterial gene function regulation across the entire microbiota, rather than in individual bacterial isolates as has been studied to date.

Workplan

Like eukaryotic genomes, bacterial genomes are subject to epigenetic modifications, specifically DNA methylation. Bacterial methylation (BACMETH) was identified in the 1960's and has historically been associated with bacterial DNA restriction-modification systems, which protect bacterial cells from foreign DNAs. More recently DNA methylation has been shown to play important roles in multiple aspects of bacterial biology, including protection against phages, timing of DNA replication, transposition and conjugal transfer of plasmids, DNA repair and cell partitioning, genome stability, and regulation of gene expression. Bacterial DNA methylation includes 3 types of modifications - N6-methyladenine (6mA), 5-methylcytosine (5mC), and N4-methylcytosine (4mC), of which 6mA is most prevalent and has been linked to gene expression regulation. Genome-wide changes in BACMETH of individual bacterial isolates have been observed in response to changes in nutrient content of growth medium, and our data in oral pathogens align with these results (Costeira et al. 2023, mSystems).

Recent developments in long-read sequencing allow for detection of multiple DNA methylation base modifications across the bacterial genome, using for example, Oxford Nanopore Technology (ONT) sequencing. However, to date only two studies have explored DNA methylation in the vertebrate gut microbiota in 2 infant human gut microbiota samples.

As part of ERC/EPSRC funded efforts we are undertaking novel characterization of BACMETH in the adult human gut microbiota. The proposed PhD project will integrate within this framework to specifically explore how host environmental exposures, such as medication use, relate to bacterial methylation in the human gut.

Year 1-2. Compare methods to quantify bacterial methylation and apply these to data from the TwinsUK cohort.

We will apply and compare multiple analytical pipelines to quantify BACMETH profiles of candidate taxa in the human gut microbiome based on ONT sequencing data. Once a consensus pipeline is reached, this will be applied to ONT data from TwinsUK cohort samples. The aim will be to characterise variability in BACMETH of candidate taxa in TwinsUK.

Year 2-3. Explore if host lifestyle and environmental factors, such as medication use, leave a signature on gut BACMETH.

The project will explore existing data on host lifestyle and environmental exposures, such as smoking, exercise, and medication use in TwinsUK. We will carry out large-scale association analyses to identify links between candidate gut BACMETH signals and host environmental factors such as medication use.

Year 3-4. Test whether bacterial methylation signatures are associated with microbially derived metabolite levels; PhD project write-up.

Candidate BACMETH signals will be compared to select microbially derived metabolites in stool and plasma samples from TwinsUK cohort participants. The aim is to identify potential functional read-outs of BACMETH.

The proposed PhD project will focus on analysis of bacterial DNA sequencing data from human stool samples from TwinsUK. The cohort is one of the most deeply phenotyped studies in the world, with multiple layers of existing biological data spanning host genetic, epigenetic, transcriptomics, metabolomic, and proteomic data, as well as gut metagenome profiles. The project will be computational, based on the analysis of novel and existing datasets.

Project Reference: MM4L13

Multi-agent systems to treat cancer – towards nanorobotics

Co-Supervisor 1:	Frederik Mallmann, Informatics
Field of Expertise:	Bioinformatics; Computational Modelling
Co-Supervisor 2:	Heba Sailem, Institute of Pharmaceutical Science
Field of Expertise:	In Vitro Modelling; Computational Modelling; Bioinformatics; Translational Medicine
Contact:	frederik.mallmann-trenn@kcl.ac.uk, heba.sailem@kcl.ac.uk

Objectives

Envision a scenario where nanobots travel through the human body, reaching a tumour and deploying their medicinal cargo to eliminate malignant cells while avoiding any unintentional damage. However, before we can successfully employ nanobots to combat cancer, we must surmount various practical and theoretical hurdles. Even for this highly specific application, a multitude of questions needs addressing: How do the nanobots locate the tumour cells? Can they assist each other? Is it safe for them to release the drug when they are 70% certain that the targeted cells are tumorous? Must all nanobots agree before taking action? What happens if some of the nanobots from the multitude malfunction? Considering the large numbers of nanobots involved, how many are likely to malfunction? Is there a risk of a collective, erroneous drug release? Given the critical nature of drug delivery within the human body, we need assurances that extend beyond heuristic predictions.

It would be imprudent to introduce nanobots into a human body based solely on their successful simulations and animal model results, without having a comprehensive theoretical understanding of their function.

Workplan

The primary objective of this project is to develop theoretical assurances that facilitate consensus within noisy and harsh environments. Other crucial aspects that need exploration include improving the precision with which nanorobots locate tumours, and enhancing the mechanisms that attract other nanorobots to the target site. Right now, the work on nanorobotics is still its infancy happens on scales that are close to the micro scale than to the nano scale. In addition, initially, we will look at very simple agents. For this reason the research topic is on self-propelling particles rather than “nano robots” – however, the ultimate goal is to lay the foundation for nano robots.

Much of the initial work will be based on the following paper: https://www.science.org/doi/full/10.1126/sciadv.1700362. Throughout the course of this PhD project, we will utilize an extensive range of methods based on mathematical reasoning. This project is truly interdisciplinary, fostering significant collaboration with biologists, chemists, and engineers. As for the prerequisites, a robust mathematical and biological background is necessary. The work carried out will be theoretical and simulation based – without wet lab work.

In addition, nanobots can be useful for targeted treatment as tumours can exhibit high heterogeneity. Your work will consider different attractants within the tumours based on microscopic images from Sailem group. Ultimately, this would allow targeting different region of tumour with most optimal treatment based on their molecular characteristics.

Project Reference: MM4L14

Multi-omics modelling to understand the genetic and molecular mechanisms of Type 2 Diabetes

Co-Supervisor 1: Kerrin Small, Twin Research & Genetic Epidemiology

Field of Expertise: Molecular Biology; Bioinformatics

Co-Supervisor 2: Oliver Pain, Basic and Clinical Neuroscience

Field of Expertise: Computational Modelling; Translational Medicine

Contact: kerrin.small@kcl.ac.uk, oliver.pain@kcl.ac.uk

Objectives

Type 2 Diabetes (T2D) and obesity-related traits are global epidemics. In the UK alone, ~4 million people are living with diabetes and 10% of the NHS budget is spent on diabetes. A limitation to early detection and treatment of T2D is its diverse clinical presentation and response to treatment. Improved methods for stratification of patients would have a large clinical impact.

Genome wide association studies (GWAS) have identified genetic variants associated with T2D, and there is much excitement in using Polygenetic Scores (PGS) to predict risk of disease from genetic data, and to identify the best course of treatment for individuals once they develop disease. Several studies have identified clinical subtypes of T2D that differ in their clinical presentation and underlying genetic signals, and there is evidence these subtypes act via molecular processes in different tissues. A key tissue for several subtypes is adipose tissue (fat), a dynamic endocrine organ.

This project will model molecular processes in human adipose tissue via multiple layers of molecular data from the TwinsUK cohort (transcriptomics, epigenetics, metabolites). It will integrate these molecular models with genetic risk of T2D to identify key molecular processes underlying disease development, and their impact on clinical symptoms.

Workplan

This project will leverage deep 'omic data derived from adipose tissue and clinical data from large biobanks to model molecular process in human adipose tissue, and use these models 1) to identify the molecular signatures of T2D subtypes in this key metabolic tissue and 2) to determine if the predictive utility of PGS are increased by joint modelling of genetic and tissue relevant 'omic data. It will involve both analyses of large datasets and exploration of new modelling techniques.

Year 1: In order to identify genes mediating risk of Type 2 Diabetes in adipose tissue we will perform Transcriptome-Wide Associations Studies (TWAS) in large biobanks, such as UK Biobank. We will examine each T2D subtype separately to determine if different genes mediate the each subtype. Given the high sex-specificity of gene expression in adipose tissue, we will run models incorporating male and female specific adipose gene expression signals. This aim will use established TWAS methods to impute the adipose transcriptome into biobanks by leveraging a new dataset describing the genetic regulation of gene expression in 2,400 adipose tissue samples generated by Co-Supervisor Small.

Year 2: Evaluation of new computational methods integrating genetic risk of Type 2 Diabetes and 'omics data. The unique molecular insights provided by large-scale TWAS can be leveraged in combination with high-resolution individual-level transcriptomic data to calculate Polytranscriptomic Scores (PTS). Recent research has demonstrated PTS can enhance prediction of disease risk and

clinical characteristics. We will develop novel approaches for the calculation of PTS models and evaluate their predictive utility for T2D and obesity-related traits.

Year 3: We will extend the methods from Year 1 and 2 into additional layers of adipose molecular 'omic data, including epigenetics and metabolomic data collected in the TwinsUK cohort. We will model each 'omic individually, and then jointly in a multi-omic framework. This aim will expand our understanding of the molecular networks that mediate the genetic risk of T2D and its subtypes.

Project Reference: MM4L15

Modelling the dynamics of gene regulatory networks controlling lineage dependent patterned cell death within developing neural circuits in Drosophila

Co-Supervisor 1: Darren Williams, Developmental Neurobiology

Field of Expertise: Animal Modelling; Development; Stem Cell Biology; Molecular Biology

Co-Supervisor 2: Alessia Annibale, Mathematics

Field of Expertise: Computational Modelling; Bioinformatics; Biomaterials

Contact: darren.williams@kcl.ac.uk, alessia.annibale@kcl.ac.uk

Objectives

Nervous systems contain a diversity of cell types intricately organised into complex functional networks. During neurogenesis developmental processes ensure that specific neuronal subtypes are generated in appropriate numbers at the right locations.

Within insect nervous systems segmentally repeated populations of 60 neural precursor cells, called neuroblasts generate the majority of neurons in the CNS. These uniquely identifiable neuroblasts divide repeatedly producing pairs of neurons, we call A and B cells. Remarkably precise 'neuroblast specific' fate-based decisions control whether the A or B neuron dies.

This patterning dictates the number of specific neuronal cell types available for building circuits and determines segment-specific and sex-specific differences in circuitry as well as evolutionary novelties in other species. This precisely patterned fate, of cell death, is controlled by two genes called reaper and grim, which are under complex transcriptional control.

Our project aims at building a mathematical model to describe the gene regulatory networks underlying these precise lineage-specific deaths, reveal key nodes within the network and then test them using sophisticated in vivo Drosophila genetics. Our goal is to uncover the 'transcriptional logic' of cell death and ultimately apply this model to other species where different patterns of cell death generate species-specific circuit motifs.

Workplan

1. The student will perform analysis of RNAseq data from doomed and non-doomed neuronal populations along with ATAC-seq and CUT&RUN chromatin profiling from the cis-regulatory regions of Reaper and Grim, the two proapoptotic genes we know to be responsible for lineage-specific cell death.

2. Machine learning tools will be used to infer gene regulatory networks that are death specific in different neuroblast lineages within the insect nervous system.

3. Non-equilibrium statistical mechanical techniques will be used to analyse the dynamics of gene expression on such network models. This mathematical modelling will allow us to predict key nodes within gene regulatory networks responsible for driving cells towards death.

4. Predicted molecular key nodes will then be experimentally tested. This workflow will include mosaic clonal loss of function experiments removing candidate transcription factors and determine changes in expression levels of Reaper/Grim and death in vivo. Alongside this CRISPR/Cas9 will be

uswed to remove binding sites in Reaper and Grim locus determine changes in expression levels and death in vivo.

5. The data will then be used for further refinement of the mathematical model and tested with application to RNASeq from a closely related wingless species where patterns of PCD are different.
https://elifesciences.org/articles/59566

Project Reference: MM4L16

A multi-omic study of Clonal Haematopoiesis-derived leukocytes post-myocardial infarction using a patient-derived xenograft model

Co-Supervisor 1:	Daniel Bromage, School of Cardiovascular and Metabolic Medicine & Sciences
Field of Expertise:	Animal Modelling; Translational Medicine
Co-Supervisor 2:	Lynn Quek, Comprehensive Cancer Centre
Field of Expertise:	Bioinformatics; Molecular Biology; Translational Medicine
Contact:	daniel.bromage@kcl.ac.uk, lynn.quek@kcl.ac.uk

Objectives

Myocardial infarction (MI) elicits a dysregulated inflammatory response that exacerbates cardiomyocyte loss, maladaptive left ventricular (LV) remodelling, and the development of heart failure (HF). Post-MI inflammation activates tissue repair but excessive inflammation exacerbates aberrant remodelling.

Clonal haematopoiesis (CH) is a novel risk factor for CV disease. Individuals acquire mutations in blood cells, including immune cells, without apparent blood disorder. Multiple large studies have identified CH as a public health risk as it is associated with increased risk of HF and death, possibly by altering the inflammatory functions of myeloid cells.

We have shown that monocytes from patients with CH, particularly those with DNMT3A mutations, are common in patients with MI. Recovery of LV ejection fraction (LVEF) was significantly worse in these DNMT3A-mutant individuals, which skew monocytes towards a pro-inflammatory phenotype. Compared to non-CH monocytes, genes associated with inflammation were upregulated, while antigen presentation and interferon responses were downregulated.

We want to study how CH affects ventricular remodelling by developing and characterising a murine xenograft MI model using CH patient-derived monocytes. Using this model, we will investigate cell-cell interactions between CH cells and tissue-resident macrophage populations, which are spatially heterogenous and known to regulate the cardiac response to MI.

Workplan

Hypotheses:

i) CH patient-derived monocytes migrate to infarcted myocardium and promote exaggerated inflammation;

ii) Inflammation varies depending on mutations and spatially restricted resident cells;

iii) CH monocytes worsen maladaptive ventricular remodelling and HF compared with non-CHIP monocytes in xenograft models.

Our research aims are:

Aim 1: Generate and characterise a CH patient-derived murine xenograft MI model.

To generate a humanised xenograft model, haematopoietic stem progenitor cells (HSPCs) from CH and non-CH individuals will be injected into NBSGW/NSG-SGM3 immunodeficient mice. Upon engraftment, mice will have circulating mutant human leukocytes, including monocytes detectable by flow cytometry (FACS). We can also detect mutations by PCR in mouse blood. Upon stable engraftment, the student will compare baseline cardiac dimensions and function using echocardiography (LV end diastolic volume, global longitudinal strain, and LVEF) in CH and non-CH mice.

Aim 2: Multi-omic characterisation of extent and quality of the inflammatory response after MI in xenografted mice.

To assess the post-MI inflammatory response and interactions of CH cells with tissue-resident populations, xenografted mice will be subjected to left coronary artery (LCA) ligation. To reveal temporal dynamics of inflammation, FACS on myocardial digests will quantify and phenotype myeloid cells in the infarct zone at 1, 3 (inflammatory phase) and 7 days (proliferative phase) post-MI. As CH skews monocytes towards an inflammatory, classical phenotype, we expect a more cellular and prolonged inflammatory response in CH mice. To resolve interactions between infiltrating CH cells with tissue-resident cells and spatially restricted effects, we will use cutting-edge, spatially resolved RNA-sequencing (Curio Seeker). The student will be trained in computational analysis of these data using established pipelines, with support of the Hub for Applied Bioinformatics (PI: Alessandra Vigilante).

Mutant monocytes from CH patients may influence 'bystander' non-mutant cells through aberrant cytokine production. The student will analyse collagenase-digested cardiac tissue of CH and non-CH mice using ELISA for IL-1b, IL-6 and CXCL2 (implicated in our previous experiments), and any other significant cytokines from spatial RNA-seq experiments.

Aim 3: Assess the impact of CH-derived monocytes on ventricular remodelling and heart failure.

To determine the functional significance of CH monocytes, the student will characterise ventricular remodelling, comparing LCA ligated or sham, CH and non-CH-engrafted mice. Cardiac function can be studied using 3D-speckle tracking echocardiography at 1 week (for early changes) and 4 weeks (the standard to observe remodelling after murine MI). After 4 weeks, excised hearts will be examined for fibrosis and remodelling markers. As mice with CH monocytes may have worse maladaptive ventricular remodelling, they will compare CH and non-CH mice after re-perfused infarction or sham to assess potential differences in infarct size. If time permits, we aim to pursue cardiac organoid models available via our collaborators in the BHF Centre of Research Excellence, to serve as a high-throughput method for targeting the novel pathways and cell-cell interactions we identify.

This project will provide invaluable knowledge on CH-mediated dysregulation of post-MI inflammation and identify potential therapeutic targets. The long-term vision is to investigate novel anti-inflammatory drugs to prevent progression to heart failure after MI.

Project Reference: MM4L17

Modelling gene regulatory network dynamics during lineage reprogramming of glia into neurons

Co-Supervisor 1: Benedikt Berninger, Developmental Neurobiology

Field of Expertise: Animal Modelling; In Vitro Modelling; Development; Stem Cell Biology;

Co-Supervisor 2: Alessia Annibale, Mathematics

Field of Expertise: Computational Modelling; Bioinformatics

Contact: benedikt.berninger@kcl.ac.uk, alessia.annibale@kcl.ac.uk

Objectives

Transcription factors can trigger the conversion of brain glia such as astrocytes into neurons, opening new avenues towards brain repair. When successful, this process appears to involve intermediate states resembling those found in natural neurogenesis. However, the process can go awry resulting in failed reprogramming. This suggests that the conversion process requires specific dynamics in order to lead to successful fate conversion.

The present project aims at developing a mathematical model describing the molecular trajectory of glial cells undergoing transcription factor-induced reprogramming into neurons. The specific goal is to determine the gene regulatory networks that define distinct stages along the trajectory and mathematically model their temporal dynamics. This in turn will lead to prediction of key nodes underlying network dynamics which will be tested by perturbation studies in experimental in vitro and in vivo models of glia-to-neuron reprogramming. Conversely, identification of these critical nodes will help determining molecular roadblocks that result in abortive reprogramming and predict molecular strategies how to overcome these roadblocks.

Workplan

1. The student will perform analysis of single cell OMICS data sets (single cell transcriptomics and chromatin accessibility) data of in vitro and in vivo lineage conversion of mouse astrocytes into induced neurons following forced expression of proneural transcription factors such as Ascl1 and Neurog2.

2. Machine learning tools (e.g. autoencoders) will be used to infer gene regulatory networks that are able to mimic the conversion process.

3. In turn, non-equilibrium statistical mechanical techniques will be used to analyse the dynamics of gene expression on such network models. This mathematical modelling will allow us to predict molecular key nodes in gene regulatory networks responsible for network dynamics and thus successful as well as abortive reprogramming.

4. Predicted molecular key nodes will then be experimentally validated by perturbation experiments of glia-to-neuron reprogramming by CRISPR/Cas9 or RNA short hairpin technology in vitro cultures of mouse astrocytes and glia-to-neuron conversion in the mouse brain in vivo.

5. This may allow for 2nd generation of single cell OMICs data (i.e., scRNA-seq and ATAC-seq following molecular perturbation).

6. The data will then be used for further refinement of the mathematical model.

Project Reference: MM4L18

Combining modelling and in vivo approaches to understand cell fate decisions in the nervous system

Co-Supervisor 1: Andrea Streit, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Development

Co-Supervisor 2: Alessia Annibale, Mathematics

Field of Expertise: Computational Modelling; Mathematics

Contact: andrea.streit@kcl.ac.uk, alessia.annibale@kcl.ac.uk

Objectives

The nervous system is arguably the most complex organ in our body with thousands of different cell types with different functions. Yet, during embryonic development, it arises from only three distinct cell populations. The neural plate gives rise to the central nervous system, while neural crest cells generate the peripheral nervous system along the entire body axis and placodal cells the sensory nervous system in the head. All three populations come from a pool of common progenitors that is set aside early in development. How do these progenitors become different from each other and acquire their unique identities?

Ultimately, the identity of a cell is defined by the genes it expresses. While the genes are critical to determine a cell's functional properties, non-coding regulatory regions are key to control where and when these genes are expressed. Thus, to understand the molecular mechanisms involved, we must integrate both gene expression and their control.

The objectives of this project are:

i) to use mathematical modelling to predict key regulators of cell fate choice based on existing large data sets;

ii) design experiments to test these predictions in vivo, and feedback these results into the model.

The project will provide new mechanistic insight into how cell identity is established in the nervous system. It will equip the student with an interdisciplinary skill set to answer fundamental questions in biology.

Workplan

This project aims to understand how neural, neural crest and placodal cells diversify. To begin to address this we have surveyed how gene expression changes from progenitor to definitive neural, neural crest and placodal cells at single cell level and identified the non-coding regulatory regions that control these changes. Now, we will combine mathematical modelling and in vivo experiments to explore the underlying gene regulatory networks and molecular mechanisms.

The first aim is to use a combination of statistical mechanical and machine learning techniques to predict a gene hierarchy as cells diversify, link transcription factors and their targets and predict how these factors drive cell fate choice.

The second aim is to design functional experiments in the chick embryo to test predictions and probe the molecular events, as well as improving the predictions made by modelling.

Together, this project will provide new understanding of the fundamental principles that govern cell fate choices. However, similar principles also lie at the heart of understanding homeostasis, disease, regeneration and repair. Our findings will therefore have a much wider impact.

The project will be co-supervised by Alessia Annibale, an expert in complex systems modelling, Grace Lu, an expert in bioinformatics of gene regulatory networks, and Andrea Streit, an expert in development of the nervous system and cell fate decisions. Throughout the project, the student will be exposed to an interdisciplinary research environment taking advantage of this supervisory team with cross-disciplinary expertise. They will be trained in different approaches for network modelling, in experimental design and development and experimental approaches to test model predictions.

Project Reference: MM4L19

Neuroimmune and immunometabolic underpinnings of autism

Co-Supervisor 1: Marija Petrinovic, Forensic & Neurodevelopmental Sciences

Field of Expertise: Animal Modelling; Molecular Biology; Development; Translational Medicine

Co-Supervisor 2: Esperanza Perucha, Department of Inflammation Biology

Field of Expertise: In Vitro Modelling; Translational Medicine

Contact: marija-magdalena.petrinovic@kcl.ac.uk, esperanza.perucha@kcl.ac.uk

Objectives

The genetic component of neurodevelopmental disorders, such as autism, is well established. For instance, mutations in the Neurexin1 gene have been linked to synaptic dysfunction in autism. However, the mutation per se is often not enough to cause the disorder, thus requiring another trigger or a "hit". Inflammation was also associated with neurodevelopmental disorders, and indirect evidence from epidemiological studies suggests that genetic mutation(s) and inflammation likely converge to disrupt brain development. Despite these observations, to date no study has directly examined how genes and inflammation together cause a shift towards atypical brain development relevant to autism, and this is the focus of this project.

Our objectives are:

1. Characterise the immune system in a mouse model of autism (Neurexin1 knockout mouse)

2. Characterise the role of maternal immune activation (MIA) in offsprings' autism development.

3. Identify new cellular and molecular pathways (both immunological and neurological) that drive the autism phenotype – in vivo (mouse model) and in vitro in induced pluripotent stem cells (iPSCs) from patients carrying Neurexin1 mutation.

By bridging the gap between cells and the whole systems, on one side, and between preclinical and clinical research on the other, this project will fill the fundamental gap in our understanding of how genetic and environmental risk factors combine to cause autism.

Workplan

Our labs have well-established in vivo and in vitro models of neurodevelopmental and immune disorders. Together, we have shown that prenatal exposure to maternal immune activation (MIA) alters immune cell populations in the offspring's brain and its development. We have also demonstrated how alterations in immune resolution (or progression of an inflammatory state) are linked to cellular metabolism in immune cells. In this project, we will combine our expertise to define the inflammatory and metabolic triggers of neurodevelopmental disorders.

Prior studies of genetic rodent models have largely examined homozygous gene mutations, but most individuals with a known genetic risk for autism are heterozygous for the risk variant, and not all will develop autism. Instead, it is thought that the risk gene confers vulnerability to exposures such as prenatal MIA. However, the timing of prenatal MIA matters. We and others have shown that an early or late gestation exposure to MIA in mice causes both over-lapping and distinct alterations in brain structure, function, and behaviour; and this likely contributes to the heterogeneity of conditions such

as autism. Therefore, in this study, we will compare postnatal neurodevelopment of mice with and without a heterozygous mutation in Nrxn1 exposed to MIA (or saline control) in early or late gestation and will compare that with findings in iPSCs derived from patients carrying the same mutation.

We will:

(1) Establish a double hit mouse model of autism. MIA will be induced during early or late pregnancy. Heterozygous Nrxn1 male and female mice (Nrxn1+/-) will be obtained by mating wild-type females and Nrxn1α+/- males. The advantage of this mating scheme is that by inducing MIA in wild-type females we will avoid the potential confound that the genetic mutation itself causes differences in the in utero environment.

(2) Characterise the immune function in pregnant females, the placenta, and the offspring.

(3) Determine behavioural deficits and their onset by performing a battery of autism-relevant behavioural assays longitudinally throughout development.

(4) Correlate behavioural phenotypes with regional brain structural indices and functional brain networks by performing longitudinal in vivo functional magnetic resonance imaging (fMRI). Highly translational technologies such as fMRI will allow us to later directly compare data obtained in this animal model with those reported in humans (data already available to us through the AISM-2-TRIALS consortium).

(5) Dissect the cellular (e.g. neuronal and microglial) and molecular mechanisms (e.g. cytokine signalling). Here, we will use electrophysiological analyses to establish the circuits and synaptic defects involved.

All the findings will further be corroborated on iPSCs harvested from patients carrying Nrxn1 mutations.

During this PhD project, students will develop both wet and dry lab skills such as: behavioural testing, fMRI, pharmacological treatments, biochemical and immunohistochemical methods, electrophysiology, immune cell isolation and culture; flow cytometry; qPCR; confocal microscopy; basic bioinformatics (i.e., analysis of RNA-seq datasets using Rstudio) and biostatistics analyses.

With this project, we will fill the fundamental gap in our understanding of how genetic and environmental risk factors combine to cause autism. This is essential to facilitate the identification of biomarkers that could improve early diagnosis; inform prevention and/or intervention strategies; and test new treatments in an etiologically valid model.

Project Reference: MM4L20

Multiscale modelling of neural crest cell behaviours in development and cancer

Co-Supervisor 1: Karen Liu, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Animal Modelling

Co-Supervisor 2: Rivka Isaacson, Chemistry

Field of Expertise: Molecular Biology

Contact: karen.liu@kcl.ac.uk, rivka.isaacson@kcl.ac.uk

Objectives

Neural crest cells are highly migratory: they travel throughout the developing embryo, eventually contributing to most organ systems and must readily adapt to dynamic changes in their microenvironment. Post-translational modifications such as phosphorylation mediate these responses through rapid and reversible control of protein activity, binding, and subcellular localisation. This project aims to decipher the mechanisms by which phosphorylation events by an important enzyme, glycogen synthase kinase 3 (GSK3) influence neural crest cell behaviours during embryo development and how they are misregulated in cancer. The host laboratories and key collaborators offer a wide range of research skills including microscopy, cell biology, biophysics and mathematical modelling. We will use an integrated combination of these techniques to identify and characterise key substrates of GSK3 at the organism, tissue, cell and molecular levels, with a particular emphasis on exploring the interaction of GSK3 with Anaplastic Lymphoma Kinase (ALK), which hyperactivates GSK3 in cancer settings.

Workplan

During the first 9 months the student will receive training from the Liu lab in tissue culture of neural crest cells and associated assays, and from the Isaacson lab on recombinant protein expression and purification to optimise production of GSK3 and ALK variants. This will set them on their way to a good set of results for the upgrade. Between 9 and 24 months they will establish proteomic profiling of GSK-3α/β in mammalian neural crest cells and use biophysics (microscale thermophoresis/isothermal titration calorimetry) and biochemistry (pull-downs) methods to determine whether ALK directly or indirectly regulates GSK-3 isomers. Subsequently, using tissue and cell methods they will build up a networked picture of interactions and phosphorylation events. This will be verified and iteratively explored at the molecular level and, through collaboration with the group of Izaak Neri in the disordered systems group in maths, will feed into a tuneable mathematical model of GSK3 signalling. In order to establish competence in the techniques the student will initially receive in person guidance for 100% of their laboratory time which will be decreased gradually. The objective is to allow the student to learn from errors and become skilled users capable of independent operation and troubleshooting. From 18 months onwards the student will continue to receive support and guidance with particular emphasis on experimental design and interpretation, dividing their time between Liu and Isaacson labs depending on their results and the directions the project takes which will be constantly monitored through lab meetings and thesis committee meetings. At such time as it is required the student will also receive training in NMR/EPR spectroscopy and X-ray crystallography from the Isaacson lab, and extensive data processing training for EM allowing them to model data at high resolution. The object is to provide sufficient room so that the student can benefit from guiding their own PhD but to provide support on an ad hoc basis.

We will encourage continual writing up of results, hopefully for papers in addition to the upgrade report so that the thesis is not a panic at the end. At the start of the final year we will formulate a plan for the thesis and remaining experimental work and we will reserve the last three months solely for thesis writing.

Project Reference: MM4L21

Embryo polarization by asymmetric gene delivery with 3D nanoneedles

Co-Supervisor 1: Ciro Chiappini, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Biomaterials

Co-Supervisor 2: Karen Liu, Centre for Craniofacial & Regenerative Biology

Field of Expertise: Animal Modelling; Development

Contact: ciro.chiappini@kcl.ac.uk, karen.liu@kcl.ac.uk

Objectives

Approaches using embryonic tissue explants and organoids have improved our ability to access, visualise and manipulate individual cells and tissues during morphogenesis and growth of the embryo. In this context, the early use of uniformly distributed morphogens as isolated stimuli to direct embryogenesis have provided valuable insights into key regulatory mechanisms directing embryonic differentiation; but, has often proved insufficient to dissect the orchestrated signalling associated with embryonic patterning.

The neural crest lineage is a transient, multipotent stem-cell like population arising in the embryo at the border of the neural plate and the non-neural ectoderm. These cells arise at the intersection of a well-defined set of morphogen gradients (Wnt, BMP and FGF), which provide coordinates within a 3D-map dictating the extent of neural crest induction. Further refinement of the map then directs the development of distinct neural crest derivatives ranging from bone/cartilage to neurons and pigment, dictated by anterior posterior gradients of the Wnt and FGF morphogens.

Existing approaches do not allow establishing the physiologically relevant gradients within organoids, nor do they allow modulation of the physical environment surrounding the tissue.

We propose to engineer a synthetic human stem-cell derived organoid using 3D nanoinjection of morphogens to mimick this neural crest “map” and to directly compare this to developing mouse embryos. The 3D nanoneedle platform for morphogen injection will allow us to finely modulate the biochemical gradients and biomechanical environment within the culture system, which will shed light on the nature and identity of neural crest derivatives.

To obtain the desired 3D morphogen distribution we will develop a platform for asymmetric and controlled gene delivery to the outer surface of embryo models. The platform will rely on nanoneedles that can selectively transfect cells to establish desired patterns and polarity(DOI: 10.1038/nmat4249). The nanoneedles will be mounted on a shape-matched device that can gently envelop the 3D cultures (DOI: 10.1126/sciadv.abf9153).

We will use this platform to selectively deliver nucleic to specific regions of explanted mouse E7 embryos and human PSC-derived embryoid bodies, inducing regional morphogen production.

We will evaluate the feasibility of this approach and its ability to generate neural border derivates including cranial neural crest and placodes.

Workplan

Based on existing protocols in collaboration with the National Physics Laboratories we will microfabricate soft shape-matched devices for 3D cultures (DOI: 10.1126/sciadv.abf9153). Using our

workflow to generate nanoneedles on flexible surfaces (unpublished data), we will decorate the devices with nanoneedles and verify their mechanical integrity. Duration 9 months

Aim 1: We will characterise the interfacing of the 3D shape-matched nanoneedle devices with explanted mouse embryos by 2-photon microscopy and electron microscopy. We will determine the impact of interfacing on cell viability and phenotype using established molecular biology assays. Duration 9 months

Aim 2: We will adsorb nucleic acids selectively on different portions of the 3D shape-matched nanoneedle device and interface it with explanted mouse embryos to selectively transfect cells on the surface. We will determine delivery efficiency using fluorescently labelled nucleic acids, and transfection efficiency by immunofluorescence. Duration 9 months.

Aim 3a: We will monitor the polarisation of the explanted embryo following selective transfection and measure phenotypical outcomes by immunofluorescence. Duration 6 months.

Aim 3b: We will adapt the protocols developed to the selective transfection of iPSC-derived embryoid bodies and measure phenotypical outcomes by immunofluorescence. Duration 9 months.

Overall this workplan will establish and validate a platform to polarize embryo models by asymmetric delivery of nucleic acids.

Project Reference: MM4L22

Cancer neuroscience: probing brain – tumour interactions in live human brain tissue

Co-Supervisor 1: Gerald Finnerty, Basic and Clinical Neuroscience

Field of Expertise: Neuroscience; Electrophysiology; Imaging

Co-Supervisor 2: Graeme Stasiuk, Imaging Chemistry & Biology

Field of Expertise: Molecular Imaging; Chemistry

Contact: gerald.finnerty@kcl.ac.uk, graeme.stasiuk@kcl.ac.uk

Objectives

Gliomas are the commonest type of tumour that start in the brain. Their prognosis is extremely poor. A major reason is that gliomas are very invasive. As a result, the current treatment, which involves brain surgery to debulk the tumour followed by radiotherapy and chemotherapy, does not extend life dramatically. New treatments are desperately needed.

Gliomas rarely spread outside the central nervous system. This suggests that there is something special about the brain environment for gliomas.

We aim to develop glioma models that give deep insights into how gliomas interact with the brain. The student will make glioma cells fluorescent with probes conjugated to quantum dots that bind to glioma specific markers on the cell surface, e.g. mutant EGFR. This will enable the student to study how the tumour cells grow and spread in brain tissue with live cell longitudinal imaging in either ex vivo "live" human brain tissue or in a rodent glioma model. Neural activity in the peritumoural cortex will be recorded electrophysiologically to determine whether brain activity affects growth of brain tumours. Finally, the student will manipulate the tumour microenvironment with existing or novel therapies to investigate their effects on glioma growth and spread.

Workplan

Year 1. Learn how to make quantum dot (QD) fluorophores emitting in the visible and NIR range and to evaluate functionalised QDs in a murine glioma model.

i) QDs

• synthesise fluorescent QDs based on InP/ZnS and Ag2S) via different methods, hot injection and microwave methodology. The nanomaterial will be coated in various ligands to prevent serum proteins from associating.

• functionalise QDs with a targeting motif for glioblastoma to create the fluorescent agent

• evaluate functionalised QDs in glioblastoma cell lines using FACS, MTS assays and confocal microscopy to show specific binding to the surface proteins on the cell. Test whether there is internalisation into the cell and if the agent has any toxic effects on the cell.

• Validate functionalised QDs on non-tumour cell lines.

ii) murine glioma model

• undertake all the Home Office training courses (module 1-4) and obtain a personal licence to handle animals and undertake the experiments.

• learn how to run preclinical IVIS scanner

• evaluate functionalised QDs in subcutaneous glioblastoma tumour models and measure uptake of the agent, biodistribution and pharmacokinetics.

Year 2: Learn how to collect blocks of live human tissue, prepare human brain tissue slices.

• The student will be trained in the Human Tissue Act and the provisions around it

• Learn how to collect blocks of live human brain tissue from the neurosurgical operationg theatre

• Incubate human brain tissue slice with quantum conjugated probes and perform live cell fluorescence imaging.

• Learn image acquisition and analysis of migrating tumour cells.

• Learn extracellular electrophysiological recording and quantification of brain activity.

Year 3: Manipulation of glioma cell growth and spread

• Use established therapies or novel agents to alter the growth and spread of glioma cells in murine glioma model and ex vivo live human brain tissue.

Project Reference: MM4L23

Deep learning approaches for deciphering cellular interactions from image data

Co-Supervisor 1:	Heba Sailem, Institute of Pharmaceutical Science
Field of Expertise:	In Vitro Modelling; Computational Modelling; Bioinformatics; Translational Medicine
Co-Supervisor 2:	Claire Wells, Comprehensive Cancer Centre
Field of Expertise:	Cancer biology; Signalling
Contact:	heba.sailem@kcl.ac.uk, claire.wells@kcl.ac.uk

Objectives

Communication between cells is essential for achieving tissue function. Cells continuously sense and align their states with their microenvironment through thousands of chemical, mechanical, or contact- dependent interactions [4-6]. Systematic decoding of complex cellular interactions and their functional relevance has proven a highly challenging problem [2]. This project aims to develop biotechnology to study interactions between epithelial cancer cells and surrounding stromal and immune cells and their association with changes in tissue architecture, exploring underlying biological mechanism.

1) Develop a deep learning and modelling approaches for inference of cellular interactions from tissue image data

2) Validate a selection of model predictions on potential cell interactions using a well established co-culture system of stromal and pancreatic cancer cells.

3) Expand the co-culture system to include three tissue compartments: immune, stromal, and cancer cells.

Workplan

Communication between cells is essential for achieving tissue function. Cells continuously sense and align their states with their microenvironment through thousands of chemical, mechanical, or contact- dependent interactions. Systematic decoding of complex cellular interactions and their functional relevance has proven a highly challenging problem. This project aims to develop biotechnology to study interactions between epithelial cancer cells and surrounding stromal and immune cells and their association with changes in tissue architecture, exploring underlying biological mechanism. Objectives 1) Develop a deep learning and modelling approaches for inference of cellular interactions from tissue image data. Histopathological images and tumour slides stained with Haematoxylin and Eosin as well as immunofluorescence markers for different tissue components (stroma, immune cells and epithelial cells) will be utilised. Various approaches including statistical and graph theory methods will be tested and visualised. 2) Validate a selection of model predictions on potential cell interactions using a well established co-culture system of stromal and pancreatic cancer cells. Genetic perturbation (e.g. CRISPR-CAS 9 approaches) and/or commercially available inhibitors that target the identified interactions will be used for validation. Findings will be fed back to the model development until satisfactory results are obtained. 3) Expand the co-culture system to include three tissue compartments: immune, stromal, and cancer cells. Again, novel computational methods for dissecting confounding factors such as cell numbers or cell proliferation related effects will be developed. Impact This project will deliver biotechnology development with

potential IP, and is thus is well aligned with the BBSRC remit. Ultimately this project will significantly advance our understanding of cellular interactions in epithelial pancreatic cells. Most importantly, it will result in generalisable methods for studying cellular interactions in various biological contexts and therefore could accelerate research on how phenotypes at the system level emerge as an outcome of interactions between different system components.

Project Reference: MM4L24

The Role of Mitochondria in Neurodegeneration Across Regions and Cell Types of the Human Brain

Co-Supervisor 1: Alan Hodgkinson, Medical & Molecular Genetics

Field of Expertise: Bioinformatics; Molecular Biology

Co-Supervisor 2: Alfredo Iacoangeli, Biostatistics & Health Informatics

Field of Expertise: Computational Modelling; Machine Learning

Contact: alan.hodgkinson@kcl.ac.uk, alfredo.iacoangeli@kcl.ac.uk

Objectives

Human mitochondria operate system-wide to regulate key biological processes. Consequently, mitochondria have been implicated in a wide range of common diseases, particularly those that occur in high energy tissues such as the brain. However, it is often not known whether altered mitochondrial function occurs because of changing cellular environments that are driven by disease states, or whether mitochondrial dysfunction forms part of the causal pathway of the disease itself. In this project we will identify whether tissue and cell type-specific mitochondrial transcriptional processes play a causal role in neurological disorders such as Parkinson's Disease and ALS. To do this, well will utilise large quantities of gene expression data from interconnected regions and cell types of human brains to computationally model the genetic mechanisms that contribute to variation in mitochondrial transcriptional processes using complex machine learning approaches, before integrating protein and metabolite data to validate downstream impacts on mitochondrial function. Validated models will then be applied to large-scale independent datasets (such as UK Biobank) to identify which mitochondrial processes are casually associated with disease. This is important to understand, as it will allow a better focus on the biological pathways that could be targeted therapeutically to reduce disease risk.

Workplan

The project will make use of existing large scale omics datasets to model the relationship between genetic data, mitochondrial transcriptional processes, and downstream functional outputs identified from protein and metabolite data from different regions and cell types of the human brain.

Aim 1: Identify genetic/molecular changes in the mitochondrial and nuclear genomes that influence key mitochondrial processes across cell types and study how they affect tissue functions. This will be achieved through processing bulk and single-cell RNA sequencing data from human samples of multiple brain regions and cell types, to extract mitochondrial transcriptional features, before identifying genetic mechanisms associated with variation in these events.

Aim 2: Based on the identified genetic/molecular signatures from aim 1, machine learning approaches will be designed and implemented to build models that are able to predict mitochondrial processes from genetic data alone. These models will then be tested in independent datasets to confirm their validity and to assess downstream biological implications using protein and metabolite data.

Aim 3: Validated models will then be used to impute mitochondrial processes into massive population and disease cohorts, such as UK Biobank (incorporating half a million individuals), and imputed values will then be compared to a range of neurological disorders and quantitative

phenotypes that are associated with disease. In this way, we will identify mitochondrial processes that are casual of disease, which could then be targeted therapeutically to modulate disease risk.