



London Interdisciplinary  
Doctoral Programme  
@ UCL, KCL, QMUL, Birkbeck, LSHTM, RVC

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## RESEARCH EXPERIENCE PLACEMENT PROJECTS

2017/18

The London Interdisciplinary Biosciences Consortium (LIDo) is one of the largest BBSRC funded Doctoral Training Partnerships in the UK and is a collaboration between University College London, King's College London, Queen Mary, University of London, Royal Veterinary College, London School of Hygiene and Tropical Medicine and Birkbeck, University of London.

We are offering 10 fully funded summer research experience placements in labs across the consortium. This special opportunity is only available to students from invited institutions and details are being sent to you following your expression of interest.

To be eligible you must be in your middle year(s) of a relevant undergraduate degree, this includes biological sciences, physical sciences, engineering, mathematics, computer science, psychology and veterinary science and be on track for at least a 2:1.

You will receive:

- £200 per week stipend
- Training, supervision and support
- A LIDo PhD student mentor
- Opportunities for networking
- The chance to win an all-expenses-paid place on the LIDo Academic Retreat in September 2018

The projects available are detailed in this booklet.

To apply please return the application form by email to [LIDo.Admissions@ucl.ac.uk](mailto:LIDo.Admissions@ucl.ac.uk) by: **Friday 9th March at 5pm**. Please make sure that your email clearly states which project you are applying for.

You can apply for more than one project, but we strongly recommend you submit separate applications tailored to each project.

Interviews will be held in late March / early April and the successful candidates will be required to attend a pre-placement briefing on Friday 22nd June. The research placements will be 8-weeks in length.

If you have any questions about the projects, application process or structure of the placements please email the LIDo Manager directly [n.mogford@ucl.ac.uk](mailto:n.mogford@ucl.ac.uk)

Good luck! We look forward to receiving your applications soon.

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## LYSOSOME EQUALITY; DO INDIVIDUAL LYSOSOMES GENERATE DISTINCT LOCAL $\text{Ca}^{2+}$ SIGNALS? (DR KILPARTICK, UCL)

Lysosomes are tiny compartments that were once perceived as uniform and to only function as the cell's recycling centre. Now, we know that lysosomes are diverse and can control cellular communication with the ion Calcium. Here, we will characterise lysosome diversity by measuring individual lysosomal Calcium signals to further understand how these structures function in health and disease.

Mammalian cells contain over 100 lysosomes that vary substantially in size, shape and subcellular position. Given this heterogeneity, I ask whether individual lysosomes generate distinct  $\text{Ca}^{2+}$  signals.

Lysosomal  $\text{Ca}^{2+}$  signalling has been implicated in numerous diseases (including Parkinson's and Alzheimer's disease) and cellular processes (such as autophagy, trafficking and the regulation of transcription). It is therefore essential that we further characterise lysosomal  $\text{Ca}^{2+}$  signalling to provide insight into the function and dysfunction of these organelles.

We will use a novel lysosome-targeted genetically-encoded  $\text{Ca}^{2+}$  indicator to:

- 1) Monitor  $\text{Ca}^{2+}$  signals at the lysosomal surface by culturing cells (skill 1), transfecting them with the indicator (skill 2) and using confocal microscopy (skill 3) to image indicator fluorescence.
- 2) Correlate  $\text{Ca}^{2+}$  signals with subcellular position by analyzing images of individual lysosomes (skill 4) as a function of their distance from the plasma membrane.

The student will acquire numerous experimental techniques, including tissue culture, transfection, confocal microscopy. In addition, they will develop invaluable scientific skills like data analysis, good laboratory practice and scientific communication. The latter will be developed by presenting data at weekly lab meetings and writing a report, in the style of a scientific publication, summarising their results.

***Cell biology, signal transduction, lysosome function,  $\text{Ca}^{2+}$  imaging, confocal microscopy.***

## RADIOBIOLOGICAL INVESTIGATION OF AN AUGER ELECTRON EMITTER RADIOPHARMACEUTICAL (DR VERGER, KCL)

Prostate cancer is the most common cancer in men. This project aims to evaluate the efficacy of a new radiotherapy targeting individual prostate cancer cells whilst not harming healthy cells. This will be done by analysing the amount of damage done to the DNA of tumours cells after their exposure to the radiopharmaceutical  $^{67}\text{Ga}$ -DOTA-PSMA.

Prostate cancer is the most common cancer in men. This project aims to develop a new Auger electron emitting radiopharmaceutical,  $^{67}\text{Ga}$ -DOTA-PSMA, by first studying its ability to induced DNA double strand breaks in prostate cancer cells. Auger electron emitting radionuclides such as  $^{67}\text{Ga}$  could offer the potential of killing tumours cells with minimal side effects to healthy tissues surrounding a tumour cell due to its specific properties (high energy radiation and very short range of energy deposition). These properties can be used to target prostate cancer cells and particularly single circulating cancer cells that are currently difficult to treat. The aim is to evaluate the therapeutic potential of  $^{67}\text{Ga}$ -DOTA-PSMA and to better understand the radiobiological mechanisms induced by Auger electron radiation exposure. Up to now, radiobiological data have mostly been acquired for external beam radiotherapies. It is important to better understand the radiobiological events in targeted radionuclide therapies also so we may optimise radiopharmaceuticals both in terms of those currently used as well as when developing new therapies.

The experimental approach consists in measuring the accumulation of a key protein involve in the repair of DNA: the histone  $\gamma\text{H2AX}$  by (semi-)quantitative techniques involving specific antibodies against it, namely by western blot or immunofluorescence microscopy. Techniques acquired will include:

1. Cell culture (DU145 PSMA+/PSMA- or LNCAP naturally expressing PSMA).
2.  $^{67}\text{Ga}$  radiolabelling of DOTA-PSMA and assessing radiochemical yield (Instant Thin Layer Chromatography).
3. In vitro assays and sample preparation: incubation with cells at different concentrations/activities and times.
4. Analysis of the amount of  $\gamma\text{H2AX}$  by immunofluorescence on coverslips and by western blotting.
5. Data acquisition: optical and fluorescence confocal microscopy (Immunofluorescence), chemiluminescence (Western Blot)
6. Data analysis: image and statistical analysis.

The student will receive full training in handling of radioactivity and in each technique.

The student will discover the interdisciplinary aspects of research in nuclear medicine and radiobiology; an increasing domain of interest in targeted radionuclide therapy. It is a good opportunity to gain experience in: radiolabelling, fluorescence microscopy and other techniques. It offers the chance to work within a cutting-edge multidisciplinary research environment. Since the methods are established, it is likely that data of publishable quality can be generated. There is also an opportunity in July to take part in the Royal Society Summer Science Exhibition on the theme of nuclear medicine.

***Radiobiology, targeted radionuclide therapy, cancer.***

## QUANTIFYING EVOLUTIONARY DYNAMICS IN MULTI-REGION SEQUENCING OF CANCER SAMPLES (DR WILLIAMS, QMUL)

It has become apparent in recent years that cancers are composed of genetically diverse populations of cells and thus should be viewed through the lens of evolutionary biology. How the cancer changes over time (i.e. how it evolves) is an important question and is relevant for predicting the prognosis of patients. This project will use evolutionary theory and next generation sequencing data to measure and quantify evolution within human cancers.

Cancer is now understood to be a process governed by evolution, where cells within the tumour acquire genetic alterations, diversify and compete for space. This intra-tumour heterogeneity has shown to be pervasive across cancer types and furthermore it has been shown to be prognostic for clinical outcomes. An understanding of the underlying evolutionary process that produces the observed ITH is likely paramount if we are to find better ways to avoid relapse and predict clinical outcomes. Due to the obvious ethical problems with following this evolutionary process unperturbed over time inferring the evolutionary dynamics is challenging. Fortunately, next generation sequencing of cancer samples provides opportunities to measure these dynamics as the genetic mutations record the evolutionary history of individual cancers. This data together with computational and mathematical approaches inspired by population genetics theory provides a powerful way to unravel these dynamics.

Recently, we took this approach and applied mathematical models to next generation sequencing from large publicly available datasets such as the cancer genome atlas (TCGA) and were able to measure the mutation rate, the fitness and time of emergence of fitter sub-populations and show that often the diversity we observed in these data had no functional impact, ie it was neutral (Williams et al 2016, Nature Genetics). This data however has some limitations, primarily that it is only single tumour samples and so there is no spatial information. Many recent studies employ a multi-region sequencing strategy where multiple samples from the same tumour are sequenced which overcomes this limitation. This projects aim will be to extend some of the methods developed for single samples to multiple samples and in particular by leveraging the spatial information we hope to be able to get a global view of the evolutionary process and also to measure spatial phenomena such as mixing of cell populations.

The approach will be to extend and develop theoretical models that include the spatial movement of cells within a tumour and observe how this changes the expected patterns of ITH. By comparing the model to data we would then be able to tease out spatial phenomena that effect ITH and measure evolutionary dynamics across the whole tumour. The project will be primarily computational in nature, using publicly available multi-region sequencing datasets and data generated within the Graham lab.

The student will gain experience in working with next generation sequencing data and in interdisciplinary approaches to integrating data with theory to extract useful insight. This will involve developing mathematical and computational approaches and fitting these models to data. The student will therefore gain skills in bioinformatics, statistics and computing.

***Cancer genomics, bioinformatics, computational biology, cancer evolution***

## GENERATION OF A KNOCK-OUT CELL LINE TO STUDY CHROMOSOME CAPTURE IN MITOTIC CELLS (DR CONTI, QMUL)

Throughout life, human cells undergo division - a process that segregates the DNA into two equal copies. This project aims to generate cell biology tools needed to understand the localisation and function of Astrin, an important regulator of cell division. In the long run, the tools created will be an invaluable resource to understanding how cells ensure normal segregation of the genome.

Chromosome mis-segregation is a cause of aneuploidy, a hallmark of aggressive cancer. Understanding the molecular mechanisms behind mis-segregation, as well as the cellular consequences, will expand our understanding of tumourigenesis and expose new cellular therapeutic targets. Our group has shown that errors in chromosome segregation can arise from defects in chromosome-microtubule attachment (Dev Cell 2004, EMBO 2006, Open Biol., 2015). My work (with graduate student Roshan Shrestha) showed Astrin/SKAP's role in stabilising chromosome-microtubule attachments using an human epithelial cancer cell line, HeLa (Nat. Comm., 2017). Using a non-transformed epithelial cell line, RPE1, another graduate student (Maddy Hart) showed that siRNA-mediated depletion of Astrin/SKAP reduces mitotic incidence, thus is infeasible. We have adopted a new strategy utilising CRISPR/CAS9 technology, to will allow us to create gene knockouts of Astrin and visualise the cellular consequence using live-cell microscopy.

The goals of this project are to use existing plasmid constructs to: (A) generate an Astrin KO cell line (B) generate a GFP-tagged Astrin cell line. The tools generated will allow the tracking of Astrin localisation and assessment of chromosome mis-segregation in live-human cells. Both project goals will expand our molecular understanding of human cell division.

A combination of cell and molecular biology techniques will be used and the goals temporally staggered to optimise time:

Goal-A: Generation of KO cell lines to assess the consequence of chromosome mis-segregation (with DC and MH; 1st - 5th weeks)

I have generated the CRISPR/Cas9 plasmid targeting the Astrin locus in human cells. To create an Astrin KO cell line, the student will make use of the haploid cell line, HAP1. I will train the student in cell culture for plasmid transfection and cell line expansion. Then long-term live-cell imaging will be carried out on KO cells (method recently standardised by MH). If we fail to generate KO cells, the student will focus primarily on Goal-B.

Goal-B: Generation of GFP-tagged Astrin cell line to track mitosis progression (With DC; 3rd - 8th week).

Together with the student, we will generate the plasmid needed to introduce GFP-cDNA into the endogenous loci of Astrin gene. The student will use the same techniques from Goal-A to generate the cell line. GFP-positive cells will be sorted using the FACS facility at the Blizzard Institute.

In addition to the excellent learning experience of being part of a interdisciplinary, collaborative and successful research environment, with frequent journal club and data presentation, the student will learn a range of cell and molecular biology laboratory techniques. Specifically the student will gain experience with the exciting and novel knockout tool of CRISPR-Cas9, as well as tissue culture techniques for human cells. Microscopy techniques will be taught to conduct phenotypic analysis once the cell line is generated.

***Our group is interested in understanding the molecular pathways that control chromosome segregation during mitosis and the consequences of errors in this process.***

## MEASURING VASCULAR RECOVERY RATE AFTER EXERCISE (DR CHARLTON, KCL)

The time taken to recover from exercise is indicative of cardiovascular risk. This project will investigate whether the rate at which blood vessels recover from exercise could be measured using smart watches. If successful, the vascular recovery rate could be used for cardiovascular risk assessment in clinical practice, and for self-assessment of vascular health in the community.

The aim of this project is to determine whether smart watches could be used to measure vascular recovery rate after exercise.

Exercise tests are widely used to assess cardiovascular health. The time taken for the heart rate to return to its baseline value immediately after exercise is predictive of long-term mortality, and can be used to assess cardiovascular risk. The time taken for the vascular system to return to its baseline state may provide complementary information on the health of the blood vessels. However, vascular recovery rate is not routinely assessed since it is much more difficult to measure than heart rate. We propose a novel technique to measure vascular recovery rate from smart watches. The technique consists of analysing the shape of the photoplethysmogram (PPG), a physiological signal which is strongly influenced by the vasculature and is measured by smart watches. We hypothesise that the vascular recovery rate assessed from the PPG will be slower in less fit subjects.

The student will use the Vortal dataset to investigate techniques for measuring the vascular recovery rate. The Vortal dataset is a benchmark dataset of physiological signals which are commonly measured by smart watches. It contains electrocardiogram (ECG) and PPG signals acquired from 40 healthy subjects before, during, and after exercise. The student will be provided with signal processing tools to extract heart rate from the ECG, and indices of vascular health from the PPG. The student will then identify those vascular indices which change independently from heart rate during recovery from exercise. The most reliable vascular index will be used to measure vascular recovery rate. Typical vascular recovery trajectories will be identified. The student will use a statistical test to determine whether the vascular recovery rate differs between more and less fit subjects. Analyses will be conducted in Matlab ®. The student will be expected to summarise their work in a brief report, using the structure of a journal article.

The student will attend the KCL Applied Maths Summer School during the project, in which they will be taught the fundamental mathematical and physical principles of engineering and design their own signal analysis tools (3 hours per day for 3 weeks). The student will also develop their ability to work in an interdisciplinary team by presenting their findings to academics and clinicians. The work is expected to form part of a publication co-authored by the student.

The stipend will be £200 per week for the 8-week project. The Summer School fee will be covered from the remaining funds.

***Biomedical Engineering: The student will use mathematical techniques to analyse human physiological data and draw clinically relevant conclusions.***



## CHARACTERISING THE EVOLUTION OF DRUG RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS (DR PHELAN, LSHTM)

The project focuses on the analysis of a large whole genome sequencing dataset in the context of drug resistance in *Mycobacterium tuberculosis*, the causal agent of tuberculosis disease. It aims to *in-silico* profile resistance through the detection of known genetic mutations. This information will shed light on the emergence of resistance to a multitude of drugs used for treatment.

Tuberculosis disease (TB), caused by *M. tuberculosis*, leads to an estimated 1.7 million deaths in 2016 alone and is one of the top ten causes of deaths worldwide. Whilst the availability of anti-tuberculosis drugs has contributed towards the reduction in prevalence of TB, a recent resurgence in the number of cases has been seen in many parts of the world. This is, in part, due to the development of anti-microbial resistance. Multi drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) occurs when the bacterium acquires genetic mutations in genes coding for drug targets or activators. Many of these genes and the causal variants have been identified through experimental allelic exchange techniques, and more recently genome wide association studies. Compensatory mutations, which offset the fitness cost incurred by causal variants, often quickly arise in a population after a drug-resistance mutation making it difficult to distinguish between them.

Next generation sequencing (NGS) allows for a whole genome to be sequenced in a fast and cost-effective way and enables the detection of all possible known resistance alleles. *TBProfiler* is a tool which was developed at our lab to provide a web-based interface to perform drug-resistance profiling from NGS data. Since its launch in 2015, over 7,000 sequences have been processed by *TBProfiler* including ~1000 MDR-TB and ~250 XDR-TB isolates. This dataset represents sequences from across the globe and has not yet been analysed in aggregate.

The WHO described the understanding of levels of background resistance as vital for the development of new anti-TB drugs. Characterising the molecular evolution of drug resistance in this large dataset will shed light on background resistance, cross-resistance and compensatory mutations among many other insights. In addition, the raw data will be reanalysed using bioinformatics approaches to identify currently unreported mutations such as large structural variants (e.g. the deletion of *thyA-dfrA* involved in ethionamide resistance). The phylogenetic reconstruction of the isolates together with ancestral reconstruction methods will be used to ascertain when a particular mutation was introduced into a population allowing for a complete picture of the stepwise acquisition of resistance seen in MDR-TB and XDR-TB. Similarly, this technique will allow for the characterisation of compensatory mutations. Additional sequencing will be performed to analyse strains not present in the dataset or to confirm the presence of a mutation.

The student will have an opportunity to work at the cutting edge of bioinformatics analysis and the overarching big-data analytics. These skills are highly transferrable both within multiple academic and non-academic disciplines. The student will have the opportunity to work with and learn from the large team of bioinformaticians in our group, as well as others working in the field of pathogen molecular biology at LSHTM. Outputs from the project will be used to write a manuscript for publication, with the student being able to contribute and gain authorship of a paper in a journal of high standing.

***This proposed work involves the analysis of Mycobacterium tuberculosis next generation sequencing data. Data will be analysed using cutting edge bioinformatics tools enabling the generation of insights into the evolution of drug resistance.***

## INVESTIGATING THE MOLECULAR MECHANISMS OF NUCLEAR DEGRADATION IN KERATINOCYTE DIFFERENTIATION (DR ROGERSON, QMUL)

The nucleus is required for cells to survive. However, skin cells need to remove their nuclei for the formation of healthy skin and a strong skin barrier. How this process is regulated is not well defined. In this project we will investigate this process and how to switch it on when impaired; it could not only restore skin barrier but also arrest cancers by preventing cell division.

In healthy skin, epidermal keratinocytes undergo removal of their entire nucleus in the formation of the skin barrier. However, how the nucleus is removed and how this removal is controlled remains to be defined. We have begun to define this process and identified small structures containing nuclear material that are formed in differentiating keratinocytes. We have also published data that supports a role for the AKT1-mediated phosphorylation of nuclear Lamin proteins being necessary for nuclear degradation. This project aims to determine whether protein machinery known to regulate other organelle membrane deformation are required to initiate nuclear degradation, and are controlled by Lamin phosphorylation.

Nuclear removal is disrupted in various common skin diseases in the young and old, eczema and skin cancers respectively, and in aged skin (xerosis), leading to nuclear retention in the skin. However, nuclear removal in keratinocyte differentiation of healthy skin is currently not well defined. Defining the process of keratinocyte nuclear removal will not only help to understand how healthy skin maintains the essential skin barrier but also indicate therapies that may help to treat eczema, xerosis and skin cancers where nuclear removal is defective. Also being able to remove the nucleus "on demand" could be a valuable treatment modality for cancers generally,

Rat epidermal keratinocyte cultures differentiate and lose their nuclei after reaching confluency. To investigate the formation of the nuclear structures a project student would undertake the culture of these rat epidermal keratinocytes. These cultured cells would then be used by the student to investigate the localisation of cytoskeletal proteins, through immunostaining of proteins such as actin and tubulin, to define the arrangement of the cytoskeleton around forming and already formed buds. The student would then determine the localisation of known membrane deforming proteins, such as BAR domain-containing proteins and others such as septins, to define whether membrane deforming proteins are localized to these forming or already formed buds. The student will then perturb the nuclear degradation process by inhibiting AKT1 mediated Lamin phosphorylation and by expressing non-phosphorylatable Lamins and they will determine the effect on the localization of cytoskeletal proteins and membrane deforming proteins.

The student would have the opportunity to work in a world class laboratory environment, giving them an important insight into the world of academic research. They will learn a variety of valuable techniques including cell culture, immunocytochemistry and confocal microscopy as well as experience the nature of planning, performing and analyzing experiments. They will also be able to interact with the rest of our department, allowing them to explore the current breath of research projects undertaken and make academic contacts which could help them form a career in research.

***In the formation of healthy skin and a strong barrier to the environment, keratinocytes of the epidermis remove their nuclei in a programmed manner, however, the mechanisms are not well understood.***

## DIFFERENTIAL GENE EXPRESSION BY ISOTYPE-SPECIFIC HUMAN MEMORY B CELLS (DR KING, QMUL)

B cells are part of the human immune system that respond to infection by making proteins called antibodies. Different types of antibodies are called isotypes, and there is some evidence that a B cell's isotype may reflect how it behaves to fight infection. Here we will examine whether isotype-specific B cells have unique gene expression patterns that explain these different properties.

In the human immune system, B cells generate antigen-specific antibodies in response to infection, and these antibodies play key roles in limiting and resolving immunological challenges. The persistence of a subset of "memory" B cells in the body can provide a memory of past infections and allow more rapid and effective responses to future challenges. During initial exposure to antigen, B cells can change antibody isotype from IgM or IgD to one of seven other isotypes (IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 and IgE). While the antigen, route of exposure and cytokine signaling play a role in isotype specification, different isotype-specific antibodies can be produced to a single antigen, with vastly different immunological outcomes. For example, in response to allergens IgE antibodies can result in harmful inflammatory responses, while IgG4 antibodies lead to immune tolerance (James et al. 2011, JACI 127 509-516). Therefore, identifying factors that direct isotype specification is essential to understand effective immunological responses. Furthermore, B cells expressing specific antibody isotypes have been reported to express varying levels of important surface markers (de Jong et al. 2017 Immuno Cell Biol 95 744; Tong et al. 2017 PNAS 114 E8411-8420), which may convey different abilities to isotype-specific B cells, particularly regarding activation, proliferation and antigen responsiveness. However, the extent to which different isotype-specific B cells demonstrate such differential gene expression has yet to be explored satisfactorily.

To investigate this important question we are currently engaged in analyzing the gene expression patterns of tonsil-derived human B cells expressing different antibody isotypes using RNA-sequencing. This will generate a list of candidate genes that are differentially expressed between isotype-specific B cells. The student will be responsible for comparing expression levels of these genes, along with genes identified in previous studies, between different isotype-specific groups of B cells using a combination of quantitative real-time PCR, western blotting and flow cytometry. Bioinformatic training will also be available to the student if they desire to interrogate the RNA-seq datasets themselves. Ultimately these experiments will lead to a better understanding of the differences between isotype-specific B cells and will be broadly relevant to a wide range of immunological contexts, including vaccination, allergies, and asthma.

This project will provide the student with the opportunity to challenge themselves with an engaging project addressing a novel and innovative question in human immunology. In addition to contributing towards a larger project that may lead to authorship on a peer-reviewed paper, the student will gain experience in several fundamental molecular biology techniques, including RNA isolation, cDNA generation and real-time quantitative PCR, as well as the opportunity to receive training in basic bioinformatics. These skills will help to prepare them for future interdisciplinary research projects.

***This study will examine how gene expression patterns reflect memory B cell identity and function in the human immune system.***

## EFFECT OF NOVEL PET RADIOTRACER <sup>89</sup>Zr-OXINE ON DNA DAMAGE AND CELL FUNCTION IN MIXED LEUKOCYTES (DR MAN, KCL)

Leukocytes accumulate at infection sites, a property exploited in medical imaging to locate infections by injecting radiolabelled cells. We are currently developing a new tracer with improved imaging characteristics. The student will use fluorescence microscopy and cellular assays to evaluate the damage caused by this radiotracers on leukocytes, providing toxicity data for future clinical studies.

The main question to be answered is how <sup>89</sup>Zr-oxine compares to <sup>99m</sup>Tc-HMPAO and <sup>111</sup>In-oxine in terms of cellular damage. Radiolabelled cells need to remain alive and functional to be useful imaging agents. Several studies (Thierens HM, J Nucl Med 1992; Stoeckli TC, Scand J Clin Lab Invest 1996; Liberatore M, EJNMI 2003) have described the toxic effects of <sup>99m</sup>Tc-HMPAO and other tracers on lymphocytes and granulocytes. For <sup>89</sup>Zr-oxine to be usable in the clinic, its toxicity towards leukocytes should be equal or lower than that of currently used radiotracers. By studying these effects, we aim to provide preliminary data that will be useful when submitting a trial authorisation to the regulatory authorities.

The experimental approach is to perform in vitro assays to evaluate the effect of <sup>89</sup>Zr-oxine on DNA damage and cell function in human peripheral blood leukocytes. DNA damage will be assessed by measuring the accumulation of the  $\gamma$ H2AX DNA repair signalling molecule in the cell nucleus by confocal microscopy. The use of cell type-specific antibodies and magnetic-activated cell sorting will allow us to distinguish DNA damage in different leukocyte populations. Lymphocyte viability after radiolabelling will be assessed by simple dye exclusion studies in culture. Granulocyte function will be assessed by cytokine release or simple chemotaxis assays. The required equipment and expertise are available in the Department with established protocols in place and the applicant has demonstrable experience in each of these techniques.

The typical experimental steps taken by the student would be as follows:

1. Radiotracer preparation and leukocyte isolation from blood samples
2. Leukocyte labelling with <sup>89</sup>Zr-oxine (controls: cells labelled with <sup>99m</sup>Tc-HMPAO or <sup>111</sup>In-oxine, unlabelled cells)
3. Sample preparation for in vitro assays
  - a. Immunofluorescence staining for  $\gamma$ H2AX and leukocyte population markers
  - b. Functional assay for granulocytes (e.g. cytokine release, chemotaxis)
  - c. Cell culture for proliferation studies of lymphocytes
4. Data acquisition: confocal and brightfield microscopy, fluorescent plate reader
5. Data analysis: image analysis (ImageJ or equivalent), Excel, statistical analysis

In practice, steps 1 to 4 are performed on the same day. Depending on the type of assay, steps 4 and 5 can be performed on following days.

The applicant will ensure that the student receives full training in each technique, particularly in the handling of radioactive isotopes and human blood.

The student will be fully immersed in our interdisciplinary School of Imaging Sciences and attend seminars and discuss their work with clinicians, biologists, chemists, engineers, and physicists. They will learn radiotracer synthesis, leukocyte isolation from blood, cell labelling, advanced microscopy, image analysis and statistics. Because the methods are established, it is likely that data of publishable quality can be generated. There is also an opportunity in July 2018 to take part in the Royal Society Summer Science Exhibition on the theme of nuclear medicine.

***Nuclear medicine, medical imaging, radiation biology, translational medicine.***

## STRUCTURE/FUNCTION STUDIES OF POINT MUTATIONS IN VOLTAGE GATED SODIUM CHANNELS RELATED TO HEART DISEASE (DR SULA, BIRKBECK)

Voltage gated sodium channels are proteins found in cell membranes. The main function of these channels is to initiate the cell electrical signaling process called the action potential. In humans there are 9 different but closely related sodium channels expressed in different tissues including the central nervous system, peripheral nervous system, heart and skeletal muscle. Mutations of sodium channels results in a various neurological and cardiovascular diseases, making these proteins important targets for development of new pharmaceutical drugs. We are interested to understanding the molecular basis of these diseases by studying the structure and function of the protein mutants. This project will focus on one conserved amino acid that when it is mutated causes a heart condition called Brugada syndrome which leads to cardiac sudden death. The structural and functional information gained in this project will help in designing and developing new therapeutics.

This project will entail creating, characterizing, and solving the structures of pathological point mutations involved in the protein stability and structure of voltage gated sodium channels in an effort to understand the molecular basis of disease.

Voltage-gated sodium channels are membrane proteins that are important for the initiation of action potential. In humans, sodium channel mutations cause a number of diseases; hence they are the targets for the development of pharmaceutical drugs. There are 9 sodium channel isoforms (Nav1.1 to Nav1.9) expressed in different tissues in humans. The prokaryotic sodium channel (NavMs) whose crystal structure I solved in the Wallace lab recently (Sula et al, Nature Comms, 2017) has been shown to be a good model studying human sodium channels. This new structure identified a new motif. Specially a tryptophan residue (W77) which is conserved in all domains of all human homologues interacts with residues in the S4-S5 linker which is associated with channel opening. Electrophysiology mutational studies of W77 showed that the function was abolished when mutated to alanine. This project will investigate the effects of a pathological point mutations (W77) in the protein stability and structure of voltage gated sodium channel NavMs, shedding light on the molecular level and structural features of the channel when this important residue is mutated. Voltage gated sodium channels are crucial components in human health and knowledge of the effect of disease causing residues should ultimately aid in the design of new therapeutics.

The wild type (WT) full length NavMs channel is produced routinely in our lab. In this project, single point mutations will be introduced into the NavMs by mutating W77 to alanine, cysteine, and phenylalanine. Once the clones are obtained they will be expressed in E. coli system. The purified protein mutants will then be characterized by circular dichroism spectroscopy to determine the relative thermal stabilities and dynamic light scattering to determine the molecular nature of the protein before it is used for crystallographic studies. These studies will be feasible during the course of the proposed studentship as we have already determined the structure of the WT protein.

The student will be trained in techniques such as molecular biology (cloning, expression, purification of membrane proteins), spectroscopy techniques (circular dichroism, dynamic light scattering) and structural biology (protein crystallography). The student will be a part of the Wallace lab, where all the members of the lab (approximately 10 students and postdocs) participate in weekly group meeting and tutorials. Towards the end of the placement the student will present their research results to the group. The student will benefit from working in collaborative research environment.

***Molecular and biophysical characterisation of voltage-gated sodium channels.***