BBSRC White Rose DTP University of Leeds PhD studentships

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We are always happy to hear from interested students!

The following projects are available this year

- Understanding the photoprotective mechanism: correlation of the structure and optical properties of single Light Harvesting proteins
- Tracking the use of energy in insect flight
- Structure and function of specialised ribosomes in the Drosophila melanogaster brain and testis
- Bionic protocells for enhanced performance of membrane proteins in biotechnology
- Dissecting the role of root exudates in density-dependent growth responses in plants
- Understanding the mechanism of TRPC1/4/5 channel activation by the natural product tonantzitlolone
- Epigenetic mechanisms underlying responses to environmental stress
- Life in the freezer – how do proteins function in the cold?
- Effects of PDE48 inhibition on excessive weight gain-induced impairment in cognitive function in laboratory mice
- Structural and functional studies on proteins required for vision
- Smart protein networks: exploiting enzyme mediated chemical cross-linking towards novel biomaterials
- Epigenetics, embryogenesis and plasticity in insects
- Spatio-temporal dynamics of resource exchange between plants and competing root symbionts
- Understanding the fusion mechanism of Herpes Simplex Virus
- MicroRNA evolution in placental mammals: Unravelling conservation and divergence in their regulatory mechanisms in early pregnancy in different placental mammals.
- Chemical tools as modulators of amyloid formation
- The in situ molecular structure of active calcium ion channels
- Targeting enzymes for the degradation of plastics
- Nanoinjection: a single molecule platform for the quantitative and targeted delivery of protein complexes into cells for functional analysis
- Biohybrids for Solar Chemicals and Fuels: Whole-Cell Photocatalysis by Non-Photosynthetic Organisms

- Protein/lipid interactions: Determinants of lipid interactions with membrane proteins investigated by machine learning, molecular simulations and mass spectrometry.
- Understanding and predicting specificity and selectivity in auxin receptor complex formation
- Floral pollen resources and their importance for pollinators and pollination services.
- A computational and mechanistic study of sodium-activated potassium channel function
- Exploring the molecular mechanisms of CREB activation in the human papillomavirus (HPV) infected epithelium
- A multi-disciplined approach to understand membrane protein dynamics
- Determination of the molecular architectures of centrosomes and basal bodies using a novel labelling method and cryo-electron tomography
- Understanding how the ABC-F proteins mediate antibiotic resistance
- Determination of the structure of protein complexes from hydrogen-deuterium exchange and mass spectroscopy
- The Tubulin Code: understanding Tubulin structure, function and organisation in the brain
- Investigating the developmental genetic mechanisms controlling the timing of body segmentation in insects.
- Cryo-EM studies of amyloid fibrils and their mechanisms of formation in vitro and in vivo
- The structure and function of the β barrel assembly machinery
- Engineering lipoglycopeptide biosynthesis to produce new antibiotics
- Inhibiting protein-protein interactions in the early stages of amyloid formation
- Defining picornaviral replication complexes by molecular virology and state-of-the-art imaging – Novel strategies for disease control
- Structural and mechanistic analysis of Chikungunya virus replicase processing
- Selective functionalisation of auricular sensory afferents to identify the pathways mediating the effects of transcutaneous nerve stimulation
- Primed for parasitism: pathogenic nematodes tailor their response to host plant exudates.
- Capturing how Hsp90 prevents the formation of cell-disruptive toxic amyloid species by Cryo-EM in a *C. elegans* model for Alzheimer's Disease
- Flight mechanics in insects
- Programming the subcellular localization of enzyme inhibitors
- Nuclease-resistant DNA nanostructures for high precision plant genome engineering
- Determining the role of molecular co-chaperones in virus infection: a novel antiviral approach
- Designer Cross-Linking Chemistry To Probe Protein-Protein Interactions in vivo
- Understanding cellular signaling networks via protein-conjugated chemical tools
- Molecular mechanisms of how human DNA damage response controls the pathway choices of DNA repair.
- Ubiquitin chain recognition by deubiquitylating (DUB) enzyme complexes
- Probe multivalent protein-glycan interactions on dendritic cell immune regulation using polyvalent multifunctional glycan-nanoparticles
- How cells respond to stress: Molecular mechanisms of the unfolded protein response.

Understanding the photoprotective mechanism: correlation of the structure and optical properties of single Light Harvesting proteins

Peter Adams Stephen Muench

Light-Harvesting Complex II (LHCII) is a chlorophyll-protein complex found in plant chloroplasts, estimated to be the most abundant membrane protein on Earth. LHCII has a primary role as the major antenna protein for absorbing solar photons and channelling energy to Photosystem II (PSII), and a crucial secondary role in protection of the system from accumulation of excess energy. This project will use cutting-edge biochemical and biophysical techniques and our world-class microscopy facilities to study how LHCII can switch between different states. Specifically this project aims to: (1) Determine a high-resolution structure of the LHCII in the “light-harvesting” vs “protected” state. (2) Quantify the effect of different protein-lipid interactions on LHCII. (3) Correlate changes in molecular structure with changes in fluorescence. (4) Generate a model for the mechanism of photoprotection. To do this, LHCII will be biochemically purified and characterized with state-of-the-art fluorescence techniques (to monitor photoprotective state) in parallel with single-particle cryo electron microscopy (for structure). You will use LHCII either isolated in detergent suspension or incorporated within nanoscale lipid bilayers to test the effect of lipids. This project would improve our understanding of this important protein and could be exploited by others to develop crops with higher yields.

Tracking the use of energy in insect flight

Graham Askew Simon Walker

Insects are amongst the most diverse, successful and economically important orders on earth and flight is key to their success. Flight is one of the most energetically expensive modes of locomotion and there are few aspects of an insect's ecology, behaviour and physiology that are not affected by its energetic demands. During all modes of locomotion, muscles convert chemical energy (ultimately derived from food) into mechanical work that is ultimately transferred to the environment to produce movement. The energetic demands of flight in insects varies with body size and between different taxonomic groups. In order to understand this variation, the transfer of energy from the level of the muscle to the environment must be tracked, quantifying the losses at each stage of the process. In this project a range of state-of-the-art techniques (including respirometry, muscle physiology and high-speed imaging) will be used, providing an unprecedented understanding of energy expenditure in this diverse and ecologically important group.

Structure and function of specialised ribosomes in the Drosophila melanogaster brain and testis

Julie Aspden Juan Fontana Amanda Bretman

The average cell contains ~10 million ribosomes, comprised of ~80 ribosomal proteins and 4 rRNAs. Until recently it was thought that all ribosomes were the same. But substantial new evidence has revealed that ribosome heterogeneity provides and additional level of translational control. These different ribosome populations are termed ‘specialised ribosomes’. How these specialised ribosomes translate specific mRNA pools remains a mystery. This project aims to understand how changes in ribosome composition alters ribosome structure and how this enables ribosomes to translate specific mRNA pools.

We have discovered differences in ribosome composition in *Drosophila melanogaster* brain and testis. mRNA translation is particularly important during sperm production and neural function so it will be exciting to understand how this novel mechanism of gene regulation is achieved and how it contributes to brain function and male fertility.

Using a cutting-edge combination of genetics, biochemistry, translatomics and structural biology this project will uncover the structure- function relationship pf specialised ribosomes. To understand changes to ribosome structures this project will involve Cryo-EM and to determine which mRNAs specialised ribosomes translate we will use Ribo-Seq. This work has potential to shed light on the underlying mechanism of human diseases caused by mutations to ribosomal proteins e.g. Diamond- Blackfan.

Bionic protocells for enhanced performance of membrane proteins in biotechnology

Paul Beales Lars Jeuken Stephen Muench

Protocells are seen as a stepping-stone to understanding the origin of life and are being developed to generate novel cell-like biotechnologies. They are typically vesicles made from phospholipids, which have a short lifespan. In this project you will use principles of synthetic biology to enhance the stability of protocells by creating hybrid bionic systems that combine advantages of lipid and polymer vesicles. Incorporation of membrane proteins will provide transport, catalytic and signalling functionalities with potential for wide-ranging applications.

You will build on recent advances in hybrid vesicles as a durable membrane protein reconstitution system: we recently demonstrated a tenfold increase in functional lifetime of a respiratory enzyme compared to conventional proteoliposomes. A wider range of membrane proteins will be characterized in hybrid vesicles, including those of interest to our industry partners. A placement at the Institute for Protein Research in Osaka, Japan is planned to work with our collaborators on photosystem I and a voltage-sensitive ion channel. Multiple proteins will also be incorporated into protocells resulting in emergent phenomena of advanced functions.

You will learn skills in expression, purification, reconstitution and functional characterization of membrane proteins. Advanced biophysical characterization techniques including confocal microscopy and cryo-TEM will be applied to gain detailed insights into the behaviour of these proteins in hybrid membranes.

Dissecting the role of root exudates in density-dependent growth responses in plants

Tom Bennett Paul Knox

Plants have a remarkable ability to perceive both their own roots and those of neighbouring plants, and to adapt their root growth accordingly. The perception of high density root environments also leads to inhibition of shoot growth, and thus may ultimately limit yield in many crop species. We currently know very little about the signals by which plants perceive or respond to each other in the rhizosphere. However, biochemical root ‘exudates’, including the hormone strigolactone, probably play a key role. This project will use the model plants *Arabidopsis thaliana*, pea and tomato to test the role of strigolactone in root density perception, and to identify novel exudates that function in plant-plant communication. To understand plant responses to root density, transcriptomic approaches will be used in Arabidopsis, coupled with reverse genetics to identify key regulatory genes. Field work will also be performed to understand how root density affects crop growth in agricultural contexts. This multidisciplinary project will involve a combination of genetics, molecular biology, transcriptomics, cell biology, physiology, ecophysiology and advanced bioimaging.

Understanding the mechanism of TRPC1/4/5 channel activation by the natural product tonantzitlolone

Robin S. Bon Stephen P. Muench Megan H. Wright

The six human TRPC proteins form tetrameric cation channels that play key roles in cellular signal transduction/integration, and their implication in human disease (including anxiety disorders, renal/breast cancer, heart failure and kidney disease) has led specific TRPC channels to emerge as potential therapeutic targets in both academia and industry. However, fundamental and translational studies require a better understanding of TRPC1/4/5 channel regulation by endogenous and exogenous factors.

This interdisciplinary project will focus on the molecular interactions of TRPC1/4/5 ion channels with tonanzitlolone (TZL), a plant-derived natural product that activates TRPC1/4/5 channels and displays sub-type specific toxicity to human cancer cells. You will use different synthetic approaches to develop covalent labelling probes based on TZL, and use these for the mass spectrometry-based identification of TZL binding site(s) in TRPC1/4/5 channels. You will then use site-directed mutagenesis in combination with cellular assays to validate and characterise binding sites in more detail. You will work closely with a chemist and a biochemist who study the mode-of-action of other small-molecule based TRPC1/4/5 modulators, as part of a larger research programme focussed on developing better understanding and treatment of cardiovascular disease and cancer.

Epigenetic mechanisms underlying responses to environmental stress

Amanda Bretman Elizabeth Duncan Steven Sait

Animals face challenges of environmental stress from many sources, such as temperature, nutrition, toxins, disease and social interactions. These stresses can be variable and unpredictable, acute or long lasting. Their impact on the individual may reduce future lifespan, reproductive output or ability to fight disease. Alternatively a mild stress may increase resilience to subsequent stress. To combat these stresses individuals can be plastic in their behaviour or physiology, but the mechanisms that underlie these processes are not well understood. The epigenome (marks on the genome that alter gene expression) is environmentally sensitive and so may be a mechanism that allows animals respond to the environment through gene regulation. Changes to the epigenome can be long lasting, so could hold the key to how a current stress alters resilience to future stress.

This project seeks to understand how insects respond to various combinations of stresses. We will use a range of species, both the standard lab model *Drosophila* fruit flies, and also animals of direct agricultural importance (Indian meal moths, bees, aphids), to find general patterns in responses. We will then manipulate epigenetic marks chemically and genetically, and use sequencing to understand how stress alters the epigenome and gene expression.

Life in the freezer – how do proteins function in the cold?

David Brockwell Anastasia Zhuravleva Lorna Dougan

Life can be found in almost every environment on Earth including hot thermal springs, highly saline lakes and acidic waterways. Life is also found in cold environments (< 15 °C, e.g. polar environments, at altitude and most of the deep oceans). Organisms adapted to life in the cold (psychrophiles) face a wide range of challenges such as increased solution viscosity, decreased diffusion rates, decreased protein synthesis rates and most importantly the exponentially decreasing rates of reaction with lower temperature. Despite this latter effect, psychrophilic enzymes maintain activity at low temperatures but the mechanism by which this feat is achieved is unclear. The aim of this studentship is to use a wealth of biophysical and biochemical methods to investigate how psychrophilic proteins maintain catalytic activity in the cold – a feat

that, if understood, would allow provide great environmental benefit by obviating the need to heat reactions in industrial and domestic applications.

Effects of PDE48 inhibition on excessive weight gain-induced impairment in cognitive function in laboratory mice

Steven Clapcote Jamie Johnston

In humans, obesity impairs cognition and produces atrophy of brain regions associated with learning and memory. Individual cognitive performance declines with increases in body mass and energy consumption. These deficits can be observed throughout life, from childhood to late adulthood. Our lab has generated mice that have a catalytic mutant form of PDE4B (Y358C) with a decreased ability to hydrolyse Camp. We previously found that these mice show enhanced learning and memory; enhanced long-term potentiation and less synaptic depression in hippocampal slices; increased dendritic spine density in the hippocampus and amygdala; and enhanced neurogenesis in the adult dentate gyrus (McGirr et al. 2016 Neuropsychopharmacology 41:1080-92). In this PhD project, you will explore the cellular and biochemical mechanisms that might underline obesity-induced changes in brain volume and cognitive function. Specifically, you will use behavioural, electrophysiological, biochemical and histological techniques to investigate the effects of high-fat-diet-induced obesity on cognitive function in wild-type mice compared with mice with the PDE4B- Y358C mutation that was previously shown to cause cognitive enhancement in lean mice fed a standard rodent diet. These experiments will increase understanding of the cellular processes underlying cognitive decline in obesity and the effects of inhibition of PDE4B upon this phenomenon.

Structural and functional studies on proteins required for vision

Joe Cockburn Colin A. Johnson Neil Ranson

Rod and cone cells in the retina detect light using an elaborate photoreceptor, allowing us to see. Development and maintenance of the photoreceptor outer segment requires proteins located at its base that form a specialized structure called the connecting cilium. Mutations in these proteins are a major cause of childhood and adult-onset blindness.

Working jointly between the Cockburn, Johnson and Ranson groups at the University of Leeds, you will use the latest cutting-edge structural biology and imaging techniques (X-ray crystallography, cryo-EM, super-resolution imaging, soft X-ray tomography, correlative light and electron microscopy) to solve structures of connecting cilium proteins and their complexes, and place these structures into the cellular context. This will provide the first molecular-level insights into the connecting cilium architecture, which will be essential to realize the full therapeutic potential of gene therapies and drugs to treat hereditary blindness and other inherited disorders associated with ciliary dysfunction.

The Astbury Centre for Structural Molecular Biology is a major hub for structural biology in the UK, with world-class facilities and a vibrant, highly interdisciplinary research environment.

Smart protein networks: exploiting enzyme mediated chemical cross-linking towards novel biomaterials

Lorna Dougan David Brockwell Michael Webb

Proteins are bionanomachines, acting in isolation or as part of larger, often complex machinery, performing their function through structural and mechanical changes. Mechanical properties are essential for biological scaffolds, where cell behaviour can be controlled by designing material scaffolds incorporating specific structural and mechanical cues. The ability to tune protein mechanics provides new opportunities to understand the role of force in biological systems, and to create bespoke scaffolds for biomaterial applications.

The aim of this studentship is to investigate the structure and mechanics of folded protein-based networks, using a combination of experimental, computational and theoretical methods. By understanding the properties of the building block (the proteins) we will have predictive control of the biomaterial. This approach will bridge the gap between single molecule mechanics and material biomechanics, revealing how the mechanical properties of individual components are translated to the properties of macroscopic materials. We will investigate a range of candidate chemical and enzymatic approaches to cross linking including the use of sortase and SpyTag/SpyCatcher to install covalent peptide and isopeptide linkages.

Epigenetics, embryogenesis and plasticity in insects

Elizabeth Duncan Andrew Peel

All animals respond to their environment but some are able generate morphologically and behaviourally distinct individuals from the same genome in response to an environmental cue, a phenomenon known as phenotypic plasticity.

Phenotypic plasticity is observed in all animals but is best characterised in insects. A classic example of plasticity is seen in honeybees where reproductive queens and sterile workers are generated from the same genome in response to nutrition early in life. Previous research has shown DNA methylation regulates this process, yet we don't understand the role of DNA methylation in embryogenesis or in other examples of phenotypic plasticity.

Pea aphids, which are an important crop pest, also exhibit plasticity; in summer aphids reproduce asexually, but as winter approaches females detect this and alter the development of their embryos giving rise to females that reproduce sexually.

In this project we will use a variety of cutting edge techniques to investigate the role of DNA methylation in normal embryogenesis in the honeybee and pea aphid and assess whether DNA methylation is a conserved mechanism underpinning plasticity.

Spatio-temporal dynamics of resource exchange between plants and competing root symbionts

Katie Field P. E. Urwin Jurgen E. Schneider

The vast majority of plant roots form mutualistic symbioses with arbuscular mycorrhizal fungi (AMF) whereby AMF supply their host plant with otherwise-inaccessible soil nutrients in return for carbon fixed through photosynthesis. However, plants rarely associate with mutualistic symbionts alone. Instead, parasitic and mutualistic symbionts may simultaneously occupy root systems, with potentially large impacts on plant growth and development. Such scenarios are particularly pertinent within agroecosystems where farmers need to balance resource trade-offs between promoting beneficial soil micro-organisms while suppressing parasites to sustainably enhance yields.

The effects of competing parasitic root symbionts on mycorrhizal carbon-for-nutrient exchange are unexplored, indeed the overarching question of whether or not plants can regulate provision of resources to "reward" beneficial partners and "sanction" parasites is hotly debated.

Together with advances in isotope tracing in CPS and PET/CT imaging available at the Experimental & Preclinical Imaging Centre (ePIC), this multidisciplinary project brings together emerging technologies across faculties at Leeds to resolve a long-standing, fundamental and pressing question: how do plants handle simultaneous, competing root symbionts? This project will use the latest technologies to investigate the temporal and spatial dynamics and mechanisms of plant resource exchange with mutualistic and parasitic root symbionts.

Understanding the fusion mechanism of Herpes Simplex Virus

Juan Fontana Neil Ranson

Herpes Simplex Virus (HSV) is a highly contagious pathogen that causes diseases ranging from skin lesions to encephalitis and neonatal infections. To infect cells, HSV, and all enveloped viruses, have to merge (fuse) the viral and cellular membranes. This process is mediated by a viral surface protein that transits from an initial, unstable conformation to a final, more stable conformation. Strikingly, there is no structure available for any herpesvirus fusion protein (gB) in its pre-fusion or intermediate conformations, and the interactions between gB and the other HSV proteins required for fusion are not well understood.

To elucidate the structure of the pre-fusion and intermediate conformations of HSV the student undertaking this project will use cryo-electron microscopy. We have previously generated a system that produces vesicles displaying full-length gB on their envelope. During this studentship. We will: (1) Generate homogeneous populations of gB in the pre-fusion or intermediate conformations. (2) Characterise the gB samples by cryo-electron microscopy. And (3) Generate simplified systems for HSV fusion, containing gB and the other glycoproteins required for fusion, and study them by cryo-electron microscopy.

Overall, this studentship will enhance our understanding of the molecular mechanisms that drive herpesvirus fusion.

MicroRNA evolution in placental mammals: Unravelling conservation and divergence in their regulatory mechanisms in early pregnancy in different placental mammals.

Niamh Forde Mary O'Connell Karen Forbes

This project brings together, in a novel manner, the research areas of placental and uterine biology, computational molecular evolutionary biology, as well as microRNA regulation to understand how miRNAs may have contributed to the emergence of placental mammals. The main focus of this project will be to undertake wet bench analysis to understand the role of phylogenetically restricted miRNAs and the genes they regulate.

Specifically this project will address three main questions:

1) Where are the miRNAs (and the genes they regulate) that arose at the time of placental mammal emergence expressed in species with different placental morphologies?
2) What genes do these miRNAs regulate and do they do this in a species specific manner?
3) Within a species, do these miRNAs regulate gene expression in a tissue specific manner?

Collectively these questions will enhance our understanding of the regulation and function of the uterus and placenta in early pregnancy in mammals that evolved different placental morphologies.

Chemical tools as modulators of amyloid formation

Richard Foster Sheena Radford

The inherent ability of proteins to aggregate into amyloid fibrils underlies more than fifty human diseases. The misassembly of soluble proteins into toxic aggregates underlies a variety of conditions including AD and Type-2 diabetes. Amylin (hIAPP) and β 2m are two proteins of interest in understanding the mechanism of protein misfolding.

The project aims to apply our considerable expertise in the protein misfolding and small molecule inhibitor fields to identify small chemical probes of hIAPP and β 2m. Such a compound will be used to provide new opportunities to understand how and why proteins form amorphous aggregates or self-assemble into amyloid and to potentially develop therapeutics to treat disease.

The project brings together our established robust assays for measuring the binding and inhibition of amyloid formation of prototype compounds and access to target expertise around the structural biology of hIAPP and β 2m proteins with distinct and complementary approaches for the identification of small molecules able to bind to and inhibit amyloid formation.
The specific aims of the project are to: (i) identify novel chemical modulators through screening, (ii) use medicinal chemistry tool and techniques to demonstrate the ability to rationally design chemical modulators of intrinsically disordered proteins, (iii) demonstrate the potential for incorporation of a structural hypothesis to binding based on *in silico* design and structural biology, (iv) optimise inhibitors for drug-likeness and pharmaceutical and pharmacokinetic properties consistent with a bioavailable agent.

The in situ molecular structure of active calcium ion channels

René Frank Nikita Gamper

In the mammalian nervous system, specialized subcellular structures including synapses mediate learning and memory. The focal release Ca^{2+} ions by ion channels is thought to be the signal that drives local, long-lasting structural remodelling within synapses. We are seeking a highly motivated PhD candidate to investigate the structural mechanism of these fundamental cellular processes.

This interdisciplinary project involves exploiting recently developed mouse genetic reagents to determine the *in situ* 3D molecular structure of calcium ion channels and to investigate activity-dependent synaptic remodelling.

The methods used will include: i) Electron tomography and computational image processing. ii) Cryogenic correlated light-electron microscopy (cryoCLEM) of synapses and thin vitreous sections. Iii) Biochemical and genetic labelling of synaptic proteins. Applications from all backgrounds in natural or physical sciences are encouraged to apply. Some experience with programming (e.g. Python, Matlab or similar) will be highly advantageous.

The University of Leeds has invested £10m in two 300keV Titan Krios electron microscopes, a high pressure freezer, and cryogenic light microscope. Thereby, the successful applicant will receive a training at the cutting edge of structural biology and molecular neuroscience.

Targeting enzymes for the degradation of plastics

Glyn Hemsworth Darren Tomlinson

The release of plastics into the environment is having well-documented, harmful effects on much of the Earth's wildlife. Plastics can be recycled but their conversion back into monomers is a significant challenge

with many currently recycled plastics having properties inferior to the starting material. Currently, there is considerable interest in exploiting biology as a source of enzymes for improved conversion of plastics back to monomer building blocks. Initial studies in this area show promise, but the enzymes being used have not necessarily evolved for plastic degradation and so further improvements are being sought after.

The plant cell wall represents a complex of natural polymers that can be degraded by microbial enzymes. A key feature of many of the enzymes involved in this process is the presence of carbohydrate binding modules which target the enzymes to their substrates. The aim of this studentship will be to exploit Affimers as specific plastic targeting domains to mimic the role played by carbohydrate binding modules. You will learn phage display, to use molecular biology to generate new protein constructs, and to use structural and biochemical approaches to study the enzymes that you generate. The enzymes generated could provide new insights into how man-made plastics could be more effectively recycled providing a pathway towards a more sustainable economy for the future.

Nanoinjection: a single molecule platform for the quantitative and targeted delivery of protein complexes into cells for functional analysis

Eric Hewitt Paolo Actis Sheena Radford

The aim of this project is to use a nanoinjection platform for the quantitative and targeted delivery of protein complexes into cells for functional analysis. The delivery of macromolecules into cells is indispensable for the study of cellular function. Whilst, nucleic acid transfection is routine, delivery of proteins, especially in biomolecular complexes, remains challenging. The nanoinjection platform uses quartz needles with ≤50nm diameter pores, known as nanopipettes, to inject macromolecules into cells. Due to the small size of the pore individual macromolecules can be detected when they are delivered into cells, thus cellular delivery can be quantified. We will use amyloid fibrils and their oligomeric assembly intermediates as model protein complexes of different sizes with which to validate the nanoinjection platform. A defined number of structurally characterised amyloid fibrils and oligomers will be delivered by nanoinjection into the cytoplasm and nuclei of cells. The effect of these protein complexes on cells will be determined using microscopy-based assays for cellular stress and viability. Thus for the first time will be able to quantify how many intracellular amyloid fibrils and oligomers are required before a cell becomes sick and dies.

Biohybrids for Solar Chemicals and Fuels: Whole-Cell Photocatalysis by Non-Photosynthetic Organisms

Lars Jeuken Kevin Critchley

Solar energy is our most abundant energy source and has enormous potential as a clean and economical energy supply. This PhD project will tap into this under-utilised source of power by engineering direct exchange of electrons between bacterial cells and inorganic photocatalysts for the biophotocatalytic production of solar chemicals such as fuels.

We have previously shown that the extracellular respiratory machinery of the bacterium, Shewanella oneidensis MR-1 (MR-1), can support direct exchange of solar energy (from synthetic photosensitisers) by transferring electrons across the bacterial outer membrane. In this project, you will use a novel synthetic biology approach to couple photocatalysts directly to this extracellular respiratory machinery. This will create biohybrid MR-1 assemblies that use intracellular redox transformations *in vivo* (metabolism) to sustain light-driven extracellular catalysis.

You will learn skills in expression, purification, reconstitution and functional characterization of (membrane) proteins and the characterization of photosensitisers, including nanoparticles such as quantum dots.

Advanced biophysical characterisation techniques including life-time fluorescent spectroscopy, confocal microscopy, bioelectrochemistry and cryo-TEM will also be used. A range of biophysical techniques related to surface modification and bio-conjugation will be used the control the interaction between photosensitisers and respiratory proteins.

Protein/lipid interactions: Determinants of lipid interactions with membrane proteins investigated by machine learning, molecular simulations and mass spectrometry.

Antreas Kalli He Wang Frank Sobott

Biological membranes, which are comprised of lipid molecules, provide a diverse chemical environment that regulates the function of membrane proteins. For that reason, changes in the interactions of membrane proteins with lipid molecules can lead to different diseases. Despite fast-growing data that describe such interactions, the molecular and chemical details of the interactions of most membrane proteins with their lipid environment remain elusive. For this project the student will use known 3D protein structures from the Protein Data Bank and molecular dynamics simulations to identify how structural motifs of different membrane proteins interact with specific types of lipids. Then, artificial intelligence (AI)/machine learning (ML) approaches will be developed to learn the interactions, to identify patterns in protein/lipid interactions, and to provide predictions for the interactions of other proteins using only the amino acid sequence. Molecular dynamics simulations and native mass spectrometry techniques will be used to evaluate and refine some of the results of the AI/ML methodology. This project combines AI, molecular simulations and mass spectrometry that are techniques in which Leeds has world-class facilities and expertise. This position would suit a student interested in interdisciplinary science with a biochemistry, chemistry, physics or computing background, or a combination of these.

Understanding and predicting specificity and selectivity in auxin receptor complex formation

Stefan Kepinski Iain Manfield

The formation of the TIR1/AFB-auxin-Aux/IAA auxin co-receptor is one of the most pivotal protein/ligand interaction events in plant biology. In promoting the association between TIR1/AFB F-box proteins and Aux/AA co-repressors, endogenous auxins regulate almost every aspect of plant development from the earliest events of embryogenesis to the control of architecture of the entire adult plant. The function of this complex is to control gene expression by regulating levels of Aux/IAA transcriptional co-repressor proteins in response to auxin; the auxin-enhanced interaction between TIR1/AFB proteins and Aux/AAs promotes the polyubiquitnation of the Aux/IAAs, marking them for destruction in the 26S proteasome.

Recent thinking about the TIR1 co-receptor complex has been dominated by a crystal structure of the complex that shows the auxin and Aux/IAA components binding to TIR1 in the same pocket. Within this pocket, auxin acts as a kind of ‘molecular glue’ to stabilise binding of the complex. Our recent work has defined a set of early interactions in the formation of the complex that are predicted to determine the specificity of TIR1-Aux/IAA interactions and also the selectivity of endogenous auxin molecules and synthetic auxinic herbicides. In this project, you would build of these exciting discoveries, learning and using techniques including nuclear magnetic resonance (NMR), surface plasmon resonance (SPR), and Cryo- electron microscopy (Cryo-EM) to address an intellectually intriguing and economically important question in structural and plant biology.

Floral pollen resources and their importance for pollinators and pollination services.

William Kunin Jane Memmott Jeri Wright

Recent pollinator losses have been linked in part to declines in floral resources. While we have demonstrated that British nectar availability declined over the past century (Baude et al. 2016), much less is known about pollen resources, which are vital to pollinator reproduction and development. We have data on pollen production for plant species that form over 95% of UK land cover, and on the pollen chemistry for many of these plants. However, to quantify pollen resources in the field we need additional data on floral longevity and phenology.

This project will fill that gap, allowing current and past pollen resources to be estimated at farm, landscape, regional and national scales for the first time. The project will also look at phylogenetic and trait correlates of floral longevity and pollen chemistry, and experimentally assess whether pollen amino-acid composition can shift with soil chemistry. Finally, the possibility of designing “bespoke” floral plantings to complement crop pollen chemistry will be tested.

This PhD project will involve a mixture of fieldwork, greenhouse experiments, chemical analysis and statistical modelling, providing a wide skill-base for future research. It will help assess the causes of pollinator declines, and test novel methods to improve crop pollination.

A computational and mechanistic study of sodium-activated potassium channel function

Jon Lippiat Antreas Kalli Stephen Muench

The sodium-activated potassium channel $K_{Na}1.1$ (KCNT1, Slack, Slo2.2) is found in neurons and its function is to conduct ions across neuronal membranes. Malfunction of this channel causes intellectual disability and severe epilepsy, for which there is no treatment. Additionally, its knockout in mice results in hyperactive pain- and itch-related neurons. It is, therefore, a potential therapeutic target for a range of neurological conditions. Despite its importance in health and disease, many aspects of its function remain poorly understood. Computer simulations provide a powerful tool that enables us to follow the dynamics of proteins and to building dynamic models of membrane proteins in a native milieu. In this study, the student will use molecular dynamics simulations (Kalli group) to study the interplay between ions, water molecules, and the pore-lining side chains of the channel, and to understand in mechanistic detail how this ion channel transitions between active and inactive states. The models derived from these simulations will be evaluated/refined experimentally in the Lippiat and Muench groups by site-directed mutagenesis and electrophysiological measurements. The student will also determine, by cryo-EM, the structure of novel conformations of $K_{Na}1.1$, such as those caused by disease-causing mutations or drug binding.

Exploring the molecular mechanisms of CREB activation in the human papillomavirus (HPV) infected epithelium

Andrew Macdonald Adrian Whitehouse

Human papillomaviruses re-wire an infected keratinocytes to drive virus replication and persistence. In so doing, they cause a number of devastating cancers in both sexes. To generate novel therapeutics it is essential to understand the complexities of the virus lifecycle. We have established a number of primary

cell culture models that allow study of the entire HPV life cycle, and coupled with clinical data we use these resources to understand the interactions between HPV and the host. In this project we will focus on the CREB transcription factor and identify its contribution to HPV replication and pathogenesis. The project will combine virology and cell biology with state of the art cell culture models to provide novel insights into fundamental biology. It will be based in the Macdonald and Whitehouse laboratories, which are internationally recognised for their work on DNA tumour viruses.

A multi-disciplined approach to understand membrane protein dynamics

Stephen Muench Christos Pliotas

Membrane proteins make up a significant part of the genome and are the target of ~30% of therapeutics and yet our structural and functional understanding often lags behind their soluble counterparts. Exciting new developments in techniques such as electron microscopy (EM) and mass spectrometry (MS) have changed the way we can study membrane protein structure and function and provide new insights into our fundamental understanding and drive therapeutic design. This project will combine EM, MS and pulsed EPR spectroscopy to probe membrane protein structure/function using cutting edge techniques and make use of the recent ~£8M investment in these facilities. Work will initially focus on the potassium-uptake CglK ion channel from *C. glutamicum*, an RCK-domain, nucleotide/Ca^{2+}-regulated integral membrane protein, which plays a role in antibiotic efflux and drug resistance. We will use CglK as a model system to investigate “RCK-domain” membrane proteins (channel and transporters), which are ubiquitous in bacterial pathogens. By understanding their catalytic cycle and the interplay between ion/nucleotide binding and potassium in- or efflux activity we are aiming to provide new insights into small molecule drug development. The successful PhD student will be trained in complementary cutting edge techniques of interest to both academia and industry.

Determination of the molecular architectures of centrosomes and basal bodies using a novel labelling method and cryo-electron tomography

Takashi Ochi Darren Tomlinson

This project is to determine exact locations of centrosomal and ciliary proteins by developing antibody-like proteins that can specifically recognise targets and by using cryo-electron microscopy.

Centrosomes play central roles in cell division by nucleating microtubules that equally divide duplicated chromosomes into two dividing cells. In addition, centrosomes are essential for generating cilia because the core structure of the centrosome becomes the base of the cilium. Since centrosomes and cilia are highly-ordered protein complexes, they must maintain correct architectures for their normal functions. Indeed, mutations on many centrosomal and ciliary genes cause abnormal development due to their structure defects. Therefore, understanding how each protein contributes to build these organelles is important. However, we know little about exact contributions of most of centrosomal and ciliary proteins to their structures. To resolve this problem, my group currently focuses on determining the structure that is shared between the centrosome and cilium.

During the project, the successful candidate will use bacterial, insect and human cells for protein, production, purification and characterisation. Also, the student will learn how to use our state-of-art cryo-electron microscopes and analyse their data.

Understanding how the ABC-F proteins mediate antibiotic resistance

Alex O'Neill Thomas Edwards Neil Ranson

Our ability to effectively prevent and treat bacterial infection with antibiotics represents one of the key foundations upon which modern medicine is built. Unfortunately, this foundation is rapidly becoming undermined by the widespread emergence of antibiotic resistance (AR), and the World Health Organization has declared AR one of the three greatest threats facing human health. The O'Neill laboratory at Leeds is actively pursuing several complementary approaches to better understand and address this phenomenon.

Proteins of the so-called ABC-F family are an important source of AR in 'superbugs' such as *Staphylococcus aureus*. Indeed, this protein family collectively provides resistance to a broader range of clinically useful antibiotic classes than any other. Until recently, the way in which these ABC-F proteins work to cause AR remained unknown. However, the O'Neill lab has now shown that they act to physically protect the bacterial ribosome from antibiotics, although the molecular mechanism by which this occurs remains to be established.

This studentship will employ biophysical techniques (principally X-ray crystallography and cryo-electron microscopy) to determine the 3D structures of members of AR ABC-F family, alone and bound to the ribosome, thereby yielding the first detailed insights into the mechanism of this family of AR proteins.

Determination of the structure of protein complexes from hydrogen-deuterium exchange and mass spectroscopy

Emanuele Paci Frank Sobott

Determining how proteins interact with other molecules is key in understanding most biological process, development of novel therapeutics and biotechnology. The project involves the development and application of a novel approach that uses advanced experimental and computational techniques. The PhD candidate will employ molecular dynamics, *ab initio* modeling of protein structure, hydrogen deuterium exchange and mass spectrometry to determine how proteins interact and design molecules that inhibit binding. The skills gained will be highly valuable for a career in the academic and pharmacological and biotechnological sectors.

The Tubulin Code: understanding Tubulin structure, function and organisation in the brain

Michelle Peckham Darren Tomlinson Christian Tiede

The brain is full of microtubules. These important structures are essential for directing trafficking of proteins, organelles and RNA from the cell body to the synapses and back again. However, the tubulin isoforms that make up microtubules are diverse, and contain many different types of post-translational modifications (PTMs), the so-called 'tubulin code'. This large tubulin diversity must be important for neuronal function, but it is unclear why and how. The goal of this project is to use novel tools (small non-antibody binding proteins called 'Affimers') that specifically recognise tubulin isoforms and/or PTMs to understand how tubulin diversity contributes to neuronal function. The project will use a range of techniques, from protein expression and purification, to super-resolution microscopy, in vitro imaging assays and Cryo-EM, to investigate the structure of pure tubulin isoforms.

Investigating the developmental genetic mechanisms controlling the timing of body segmentation in insects.

Andrew Peel Elizabeth Duncan Ian Hope

The arthropods (flies, beetles, spiders) have obvious visible repeating body units, while vertebrates exhibit internal segmentation in the form of vertebrae/ribs. Dr Andrew Peel's past work has helped show that the genetic networks underpinning segment formation in arthropods and vertebrates share striking mechanistic similarities. In both groups, repeated structures form under the control of a 'segmentation clock'. This project will examine whether further mechanistic similarities exist. Dr Andrew Peel's recent work has helped identify segmentation 'timing factors' that regulate the spatiotemporal progression of segmentation in both a fly (*Drosophila*) and a beetle (*Tribolium*). The project will study the function of these factors in a range of insect species to see if they constitute an ancestral and conserved insect mechanism for controlling the timing of segmentation. Interestingly, these factors might play equivalent roles in vertebrates. Extensive similarity with vertebrates would make *Tribolium* a good model for understanding the human segmentation clock and how our vertebrae form. Given that arthropods and vertebrates diverged very early in animal evolution, extensive similarity might also indicate an ancient origin for segmented body plans, with many animals having lost segments (e.g. molluscs). The project therefore might offer insights into the morphological evolution of most animal lineages.

Cryo-EM studies of amyloid fibrils and their mechanisms of formation in vitro and in vivo

Sheena Radford Neil Ranson

Amyloidosis is a pathological condition associated with the aggregation of proteins into fibrils, and is the underlying pathology in diseases such as Alzheimer's and Parkinsons diseases. Despite the importance of this process to diseases that shape today's society, therapies remain remote.

In this project we will use state of the art imaging technologies to gain fundamental biological insight. Specifically, we will use the Titan Krios cryo-EM microscopes in Leeds to determine the structure of amyloid fibrils formed from the protein β_2-microglobulin and natural variants which cause enhanced amyloid disease. Using biochemical and biophysical assays, combined with cryo-EM, we will determine how amyloid fibrils form and how they bind essential cellular components including molecular chaperones. Finally, you will use cell biology, super resolution imaging and cryo-ET and cryo X-ray tomography to examine fibril formation within living cells.

Overall, therefore, the aim is to provide new mechanistic insights into fibril structure and fibril-induced cellular disruption by exploiting modern cryo-EM to the full.

The structure and function of the β barrel assembly machinery

Neil Ranson Sheena Radford

Anti-microbial resistance is a major threat to human health in the 21st Century, and finding targets against which we can develop new therapies that overcome growing resistance to existing antibiotics is an urgent, unmet need.

In this project we will use state of the art cryo-electron microscopy to generate new insight into the structure and function of a membrane protein complex that is essential for viability and pathogenesis of some the most serious bacterial pathogens. We will use the state-of-the-art Titan Krios microscopes in Leeds to do single-particle cryo-EM, and determine the structure of the *E. coli* β-barrel assembly machinery (or "BAM"

complex) to atomic resolution. We will also determine the structures of BAM bound to one of a range of natural binding partners that modulate function, and to neutralizing antibodies.

The overall aim is to provide new mechanistic insights into membrane protein biogenesis, discover new routes to novel anti-biotics, and provide training in state-of-the-art structural biology methods.

Engineering lipoglycopeptide biosynthesis to produce new antibiotics

Ryan F. Seipke Glyn R. Hemsworth Michael E. Webb

There is an urgent need for new antibiotics to combat antimicrobial resistance. Most antibiotics originate from *Streptomyces* bacteria, however the low hanging fruit from this resource has been picked. Genome sequencing projects have revealed that an average actinomycete harbours ~30-50 biosynthetic pathways, but unfortunately the majority of these are not expressed in the laboratory. The promise that these silent or cryptic metabolites hold has ushered in a genomics-driven renaissance in natural product antibiotic discovery.

In this project, you will characterise key steps in the biosynthesis of one such cryptic antibiotic, a novel lipoglycopeptide which we have discovered after activation of one of these biosynthetic pathways. You will use structural approaches to characterise the key glycosyl-lipid transferase that installs an essential lipidated sugar and use this to guide rational engineering of the enzyme to change the sugar and lipid components of the metabolite. Using this structure-activity relationship you will identify the antibiotic with the highest activity against clinical isolates of multidrug-resistant *Staphylococcus aureus.*

Inhibiting protein-protein interactions in the early stages of amyloid formation

Frank Sobott Sheena Radford

Amyloidosis is a pathological condition associated with the aggregation of proteins into fibrils. Despite the importance of amyloid diseases in today's society, therapies remain remote, due to a lack of understanding of some of the fundamental molecular processes involved.

In this project we will use directed evolution, biochemistry, native mass spectrometry and other biophysical assays, to develop new inhibitors of amyloid formation and to determine their mechanism of action in structural detail. In parallel, cell biology will be used to determine whether ligands that bind the proteins of interest also inhibit cytotoxicity. The project will focus on amylin (IAPP), involved in type II diabetes, and Aβ involved in Alzheimer's disease, two of the major diseases challenging today's society and for which there are currently no effective therapeutics on the market.

The student employed will learn a variety of skills in this multi-disciplinary project that, together, will open the door to new understandings of how and why amyloid fibril formation kills cells and whether small molecules can ameliorate or even inhibit this deadly process.

Defining picornaviral replication complexes by molecular virology and state-of-the-art imaging – Novel strategies for disease control

Nicola Stonehouse Morgan Herod Dave Rowlands

Picornaviruses are responsible for a number of serious diseases, including polio and foot-and-mouth disease, FMDV. There is an urgent need to develop new therapeutic strategies to address the continuing issue of picornavirus infection. FMDV is an extremely important animal pathogen- the 2001 UK outbreak

cost several billion pounds. The project aims to study the features of the viral genome responsible for both rapid replication and persistence, using a replicon system. The long-term aim of the work is to utilise our knowledge of the molecular details of replication in the development of new strategies of disease diagnosis and control.

This interdisciplinary project includes other UK institutions as well as the BBSRC Pirbright Institute and will involve close collaboration and research visits to partner institutions.

Structural and mechanistic analysis of Chikungunya virus replicase processing

Andrew Tuplin Juan Fontana Stephen Muench

Chikungunya virus is a mosquito- transmitted arbovirus that re-emerged as an epidemic in 2005 around the Indian Ocean, before spreading across Asia, Africa, Europe and the Americas. It continues to spread across regions harbouring its mosquito vector- including much of North America and Western Europe. Chikungunya virus causes acute 'Dengue or Zika like' symptoms and chronic, debilitating musculoskeletal pain with neurological complications.

This project will use cutting edge molecular virology, cryo-electron and correlative light microscopy methods to investigate how processing of Chikungunya virus non-structural proteins, within its replicase complex, control replication and expression of the viral genome. There are no vaccines or antiviral therapies for Chikungunya virus infection. Consequently, the longer- term goal of this research is to provide greater understanding of fundamental aspects of the virus replication cycle, in order to explore their potential as novel therapeutic antiviral targets.

Selective functionalisation of auricular sensory afferents to identify the pathways mediating the effects of transcutaneous nerve stimulation

Bruce Turnbull Jim Deuchars

Transcutaneous vagal nerve stimulation (tVNS) is emerging as a non-invasive therapy for many disorders including epilepsy, depression and anxiety, but there is little understanding of how it works as even the initial underlying neuronal pathways are not known. In this project we aim to understand which parts of the central nervous system mediate the effects of the tVNS process. Our approach will be to use neuronal tracers to deliver proteins into the cell bodies of the afferent neurons which lie a long way from where the vagal nerve is simulated. The delivered proteins will switch on genes that will enable identification of which sites in the CNS are important for the effects of tVNS. The project will involve a combination of molecular biology, protein chemistry, cell biology & neuroscience.

Primed for parasitism: pathogenic nematodes tailor their response to host plant exudates.

P.E. Urwin Katie Field

All parasites need to feed from their host in order to survive and they must adapt to maximise parasitic on varied hosts. Plant-parasitic nematodes are important agricultural pests, however little is known about the molecular mechanisms underpinning host preference and differential host success. We found that certain genes are induced in a host-specific manner when a plant-parasitic nematode detects host root exudates.

The nematode is “primed” before it physically encounters the root with expression of genes important for parasitism tailored to the identity of the immediate host.

This project will use Nextgen sequencing to explore the extent of “primed” gene expression in plant-parasitic nematodes and how this varies with plant identity. The role of differentially regulated genes in parasitism will be characterized using techniques including *in situ* hybridization, RNAi knockdown and genome editing of host plants. A metabolomics approach will determine components of root exudate responsible for priming.

Mycorrhizal fungi may influence root exudate components that are important for nematode priming, so their effect on nematode gene expression and subsequent parasitic success will be established.

This project will provide insights into how plant exudates could be manipulated to reduce the burden of parasitic nematodes on crop production.

Capturing how Hsp90 prevents the formation of cell-disruptive toxic amyloid species by Cryo-EM in a *C. elegans* model for Alzheimer’s Disease

Patricija van Oosten-Hawle Neil Ranson Eric Hewitt

Stress and aging challenge the health of a proteome and increase susceptibility to protein conformational diseases, a hallmark of many neurodegenerative diseases, including Alzheimer’s Disease. But how and when do amyloid proteins exert their toxic effect to cells that lead to disease in an organism? And how can we prevent their formation? This project addresses both these questions by combining biochemical and structural biology methods with high-resolution Cryo-EM imaging of the toxic species formed in an in vivo Alzheimer’s disease model. Using a *C. elegans* Alzheimer’s Disease model, our lab has recently shown that activation of Hsp90 expression prevents the formation of toxic amyloid protein deposits in the animal throughout aging (O’Brien et al, Cell Reports 2018). The student will image the progression of amyloid aggregates as the animal ages and correlate Aβ fibril formation with cytotoxicity. Aggregates formed in vitro and ex vivo will be analysed to understand their interaction with Hsp90 and their cellular toxicity analysed in combination with gaining high resolution structures by Cryo-EM.

The student will gain highly interdisciplinary training that combines the novelty and high-resolution power of Cryo-EM with capturing toxic species in an in vivo model of Alzheimer’s disease, using C. elegans as a model system.

Flight mechanics in insects

Simon Walker Graham Askew

Insects are the most agile and manoeuvrable of all flying animals. However, studying their flight presents a complex challenge. In the time that it takes a human to blink, a blowfly can beat its wings 50 times, powering and controlling each wingbeat using numerous tiny muscles - some as thin as a human hair. The aim of this project is to understand how insects control their wingbeat and sense aerodynamic forces through the subtle use of these muscles.

The PhD student will use a range of state-of-the-art imaging techniques, including macrography, multi-camera high-speed setups and CT scanning to record insects during flight. This will create an unprecedented view of the insect flight motor that will be important for understanding the evolution of flight and for the design of bio-inspired micro air vehicles that aim to replicate animal flight.

Programming the subcellular localization of enzyme inhibitors

Michael Webb Daniel Ungar Bruce Turnbull

The generation of enzyme isoform-specific inhibitors is a major challenge for medicinal chemists. In this project, you will take an alternative approach to this challenge to develop spatially-targetted inhibitors. Many of the enzymes are localized to particular compartments in the cell, by delivering the inhibitor to each compartment you will develop a general strategy to make spatially-selective inhibitors. Using oligosaccharide biosynthesis and tailoring the Golgi as a model you will use a combination of synthetic chemistry, protein chemistry and cell biology to develop small-molecule-protein hybrids and test their function in a cellular context. Methods to be used include bioconjugate chemistry as well as advanced cell biological methods, such as mammalian cell culture, fluorescence microscopy and mass spectrometry.

Nuclease-resistant DNA nanostructures for high precision plant genome engineering

Chris West Matteo Castronovo

The recent development of targeted modification of plant genomes heralds a new era in biotechnology for the 21st century. This project will develop new approaches for plant genome engineering based on nanotechnology to design DNA structures that promote genome integration at a targeted site. This technology will be combined with CRISPR-Cas9 nucleases, a biotechnology tool that is revolutionizing modern biology and medicine. The application of nanotechnology to CRISPR-Cas9 mediated gene targeting has the promise of high throughput precision engineering of the plant genome, key to the development of synthetic biology and the new generation of crop plants. These biotechnological approaches will be essential if we are to meet the demand required by the growing world population for sustainable increased food and energy production against the challenges of climate change, limited land for cultivation and increased pressure on natural resources.

Determining the role of molecular co-chaperones in virus infection: a novel antiviral approach

Ade Whitehouse Richard Foster

Viruses are associated with approximately 10-15% of human cancers, resulting in about 2 million new cases every year in the world. Research in the Whitehouse laboratory determines how viruses cause cancer and in collaboration with the Foster laboratory develops novel antiviral strategies to prevent infection and tumourigenesis. This project focusses on molecular chaperone pathways which are essential for protein homeostasis, particularly in cancers. For oncogenic viruses, molecular chaperones function as broad host factors required for viral protein folding and stability. Therefore viral proteins are exquisitely sensitive to perturbations in chaperone-related pathways, presenting a novel antiviral target. We have exciting data showing that the molecular co-chaperone, STIP1, is essential for the replication of the oncogenic virus, KSHV. This project will determine the role of molecular chaperones in KSHV biology and determine if inhibiting molecular co-chaperone function is a potential therapeutic approach for the treatment of this important human pathogen. This exciting multidisiplinary project will utilise cutting-edge methodology including quantitiative proteomics, cell biology and medicinal chemistry.

Designer Cross-Linking Chemistry To Probe Protein-Protein Interactions in vivo

Andy Wilson Sheena Radford

A key problem in life-sciences research is to understand cellular processes with molecular and temporal resolution- this would allow the identification of the transient intermediates that play key roles in the function of biomacromolecular machines, signalling, translocation and folding. The goal of this project is to develop covalent cross-linking reagents that possess (1) suitably reactive groups for high- yielding cross-linking over a variety of timescales and (2) handles (fluorophores, affinity groups) for analyses in cells. We will then use these reagents to study the interactome of outer membrane proteins (OMP's) the beta-barrel assembly machinery (BAM) and relevant chaperones of Gram negative bacteria. The results will open the door to new methods for delineating molecular reactions in cells, in general, as well as to elucidate how OMPs fold- a question of critical importance and utility in the drive to develop new antimicrobial agents that target this pathway.

Understanding cellular signaling networks via protein-conjugated chemical tools

Megan Wright Darren Tomlinson Michelle Peckham

Proteins form spatially organized, dynamic complexes in cells, giving rise to signaling networks essential for maintaining cellular function. In this project, you will develop new tools for directly labelling proteins in their native cellular environment. Our approach uses Affimers (small antibody alternatives) to direct the transfer of labels from a chemical tool to a target protein. You will design and synthesise tools that exploit different transfer chemistries and labels, and express and purify Affimers that bind target proteins implicated in cancer. This toolset will be used to track proteins via live cell and super-resolution imaging, and to tag proteins and their interacting partners for isolation and analysis by proteomics. You will apply this platform to analyze proteins central to signaling networks that are dysregulated in cancer.

For this interdisciplinary project, you will join an ongoing collaboration of three groups with expertise in chemical biology (Dr Wright), protein engineering (Dr Tomlinson) and super-resolution imaging (Prof. Peckham). This project would ideally suit a candidate with synthetic chemistry skills and a strong interest in applying chemistry to biological problems.

Molecular mechanisms of how human DNA damage response controls the pathway choices of DNA repair.

Qian Wu Neil Ranson

Life is full of decisions! One of the biggest decisions cells need to make is how to deal with DNA damage. We study DNA-double strand breaks (DSB), which are the most toxic type of DNA damage in cells. We want to understand how different proteins assemble at the sites of DNA damage, and how this allows cells to decide between different specific repair pathways. To achieve this goal, we combine cutting-edge techniques such as single-molecule methods and cryo-EM to visualize their structures and characterize their functions. This study will expand our fundamental understanding of pathway choice in DNA repair at a molecular level in healthy cells, but the long-term applications of this knowledge will be to understand how these decisions go wrong in cancer cells. Ultimately, we want to exploit these differences to develop drugs that can kill cancer cells specifically.

We are looking for an ambitious and enthusiastic student to join our research group. Successful PhD candidate will become an expert in protein purification, complex biochemical reconstitution/characterization and structural determination.

Ubiquitin chain recognition by deubiquitylating (DUB) enzyme complexes

Elton Zeqiraj Darren Tomlinson

A studentship to study Ub signalling is available in the laboratories of Dr Elton Zeqiraj and Dr Darren Tomlinson at the University of Leeds. Ubiquitylation of proteins is a post-translational signal that regulates virtually all cellular processes through the precise spatial and temporal control of protein stability, activity or localization. As such, enzymes that perform ubiquitin chain cleavage (called deubiquitylases or DUBs), are frequently mutated in disease and important drug targets in cancer, autoimmune disease and neurodegeneration.

The studentship offers a unique opportunity to study multimeric DUB enzymes in complex with their substrates by cryo-electron microscopy (cryo-EM). The student will also perform state-of-the art protein engineering work to generate tools to study DUB localization and their enzyme activity and inhibition.

The project will be conducted at the Astbury Centre for Structural & Molecular Biology at the University of Leeds. The Astbury center offers a vibrant research environment and state-of-the art infrastructure for structural biology, protein engineering, drug discovery, chemical biology and proteomics.

Probe multivalent protein-glycan interactions on dendritic cell immune regulation using polyvalent multifunctional glycan-nanoparticles

Dejian Zhou W Bruce Turnbull Yuan Guo

Cancer and allergy affect hundreds of millions people worldwide. They are directly linked to immune dysregulation: hypersensitivity to harmless substances causes allergy, but failure to take defensive action allows tumour to grow. Dendritic cells (DCs) can discriminate self and foreign substances and *instructs T cell immune response* via its surface receptors, e.g. DC-SIGN to recognise specific *glycan patterns.* Pathogens can target DC-SIGN to induce immune suppressive signals to assist infection, but the underlying mechanism is poorly understood. It is difficult to develop multivalent glycans for specific DC-SIGN targeting due to unknown tetrameric structure.

We will address this challenge by constructing tetravalent glycan (TVG) ligands on mutant DC-SIGN scaffolds to ensure perfect spatial match and specific targeting. We will conjugate multiple TVGs onto magnetic nanoparticles (MNPs) as pathogen mimetics and study their interactions with DCs. We will tune TVG-DC-SIGN binding affinity, density and inter-TVG spacing to reveal how these control DC-SIGN clustering, interacting with intracellular signaling proteins and cytokine production. Combining these results will elucidate how extracellular glycan stimulation is translated to regulate DC immune response. This knowledge is very important, allowing us to modulate DC to produce desired immune responses to develop effective immunotherapies against cancer, allergy and other diseases.

How cells respond to stress: Molecular mechanisms of the unfolded protein response.

Anastasia Zhuravleva Richard Bayliss Frank Sobott

The endoplasmic reticulum (ER) is a specific cellular site of synthesis, folding and modification of secretory and cell-surface proteins. The ER protein quality control system ensures that the newly synthesized proteins are properly folded into their native structure. Accumulation of misfolded protein in the ER results in ER stress that triggers an adaptive unfolded protein response (UPR). The link between incorrect regulation of the UPR and many devastating diseases are well known, but much remains to be learned about molecular mechanisms of the UPR. The main goal of this project is to characterize the molecular mechanism of UPR signaling and elucidate how different pathological and physiological stresses affect this complex multicomponent signaling cascade using a multidisciplinary approach that combines molecular biology (prokaryotic and eukaryotic protein production), the state-of-the-art structural techniques (nuclear magnetic resonance and mass spectrometry), and computational methods to address this challenging biomedical problem.

We are looking for an enthusiastic and ambitious PhD student with a strong interest in structural, computational and cellular biology. The successful candidate will be based at the Astbury Centre of Structural Molecular Biology and have access to our world-leading NMR and MS facilities.