BiPAS CDT - projects

The (un)structural biology of protein-RNA recognition unravelled by high-resolution HDX-MS guided modelling

Project ID: 2020_001

1st supervisor: Antoni Borysik (Department of Chemistry)
2nd supervisor: Maria (Sasi) Conte (Randall Centre for Cell & Molecular Biophysics)

Project type: Computational/experimental

Project Overview:
In this project the student will apply new high-resolution modelling techniques based on hydrogen deuterium exchange mass spectrometry (HDX-MS) to understand protein-RNA recognition. Borysik is developing a range of novel web-based tools for HDX-MS including HDXmodeller which permits the characterisation of protein solvent exchange in full resolution. We expect these tools to be very powerful particularly for proteins that are challenging for conventional methods such as those that contain significant disorder. This studentship is a great opportunity for the right candidate to pioneer the application of these methods on an important protein system that has thwarted characterisation by classical techniques.

Project Aims and Description:
The Borysik Research Group is developing a range of computational tools for advanced hydrogen deuterium exchange mass spectrometry (HDX-MS). These tools will represent the world's first online webserver for high-resolution HDX-MS and the first time that constrained optimisation methods have been successfully applied to this technique. The methods have not yet been published but the webserver is fully operation and can be located here https://hdxsite.nms.kcl.ac.uk/ with site registration available after publication of the associated research article.

The extent to which proteins are protected from HDX provides valuable insight into their folding, dynamics and interactions. Characterised by MS, HDX benefits from low protein size restrictions, exceptional throughput and sensitivity but with the consequence of a loss in resolution. Exchange mechanisms which naturally transpire for individual residues cannot be

accurately located or understood because amino acids are characterised in differently sized groups depending on the extent of proteolytic digestion. HDXmodeller is the world's first online webserver for high-resolution HDX-MS and returns high-resolution exchange rates quantified for each residue for low-resolution input data typical of the technique. HDXmodeller also returns a set of unique statistics that can correctly validate exchange rate models to an accuracy of 99%. Remarkably, these statistics are derived without any prior knowledge of the individual exchange rates and facilitate unparallel user confidence and the capacity to evaluate different data optimisation strategies.

The student will use advanced HDX-MS methods to characterise the interactions between La-related proteins (LARPs) and RNA. HDXmodeller will be applied to experimentally obtained datasets to pinpoint each residue in the proteins and quantify their extend of disorder. LARPs act on post translational cellular processes by binding and stabilizing mRNA transcripts and potentially controlling the length of polyA. The Conte lab is internationally recognized for expertise in RNA binding proteins (RBPs) and has extensive experience in investigating macromolecular structure, function and interactions. Conte recently discovered a novel protein-RNA interaction in LARPs mediated by intrinsically disordered N-terminal regions in the proteins. Interestingly, recent bioinformatics investigations showed these regions to be present in ~50% of RNA binding protein sequences suggesting a common mechanism in RBPs utilising protein disorder. The current aims for the Conte group are to define the structural basis for RNA recognition by several LARPs, including LARP6, LARP4A and LARP4B. Of particular interest is the role of protein disorder in RNA recognition which is the question at the centre of this studentship.

References:

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Structural insights in the design of protein-based biomaterials

Project ID: 2020_002

1st supervisor: Alex Brogan (Department of Chemistry)
2nd supervisor: Sherif Elsharkawy (Department of Oral, Clinical & Translational Sciences)

Project type: Experimental

Project Overview:
Protein-based biomaterials are increasingly sought after for a multitude of applications ranging from industrial biocatalysis to tissue engineering. Protein-based materials have significant advantages over synthetic materials such as improved biocompatibility (for tissue engineering) and energy efficiency (for biocatalysis). Furthermore, incorporating proteins into materials can bring additional function and capacity such as improved robustness and enhanced activity. Key to the success of protein-based biomaterials is maintaining and controlling the structure of the biomolecule. This project will explore the optimization of protein-based biomaterials for a variety of applications through a systematic and comprehensive biophysical study of protein structure, stability, and function.

Project Aims and Description:
Both the Brogan group and the Elsharkawy group use proteins as the fundamental building blocks for biomaterials for industrial biocatalysis and tissue engineering respectively. Whilst these applications may seem far apart, the success of both depends on a deep understanding of how protein structure controls properties and dictates function. Particularly, we are interested in how we can use and control protein structure to yield materials with superior properties. Critical to this will be a detailed biophysical analysis of protein structure, stability, and function throughout the design and synthesis of new biomaterials. These studies will primarily be spectroscopic in nature, using circular dichroism and FTIR to monitor secondary structure, and UV/Vis spectroscopy to assess enzyme activity. These will be complemented by dynamic light scattering and small-angle scattering techniques (SAXS/SANS) to inform on how global protein architecture is affected by biomaterial development.

Being supervised by both Dr Brogan and Dr Elsharkawy will give the PhD student undertaking this project a broad range of options to choose where to

take their project, once the core elements of protein structure determination and biomaterial design have been mastered. In the Brogan group, we have recent successes in demonstrating that surface modification of enzymes to yield protein-rich biofluids can significantly enhance enzyme activity in anhydrous conditions. This has allowed us to demonstrate that we can shift the optimal window of enzyme activity far beyond what is capable in water in terms of temperatures, activity, and substrate choice. In another application, we have shown that it is possible to modify the filamentous bacteriophage M13 for the design of new functional soft materials for potential use in biocompatible wearable technology and soft-robotics. In the Elsharkawy group, we focus on biomineralised tissues and developing bio-inspired hierarchical materials for various biomedical applications. The research group is leading the development of ambitious projects that exploit intrinsically disordered proteins to design and tune organic materials, to control crystal nucleation and hierarchical mineralisation at multiple length-scales. Our goal is not only to develop materials for tissue regeneration, but also looking into the protein-mediated physicochemical mechanisms that drive biological hard tissues during developmental and disease progression.

Overall, the core aims of this project will be a biophysical centred investigation into the development of the next generation of protein-based advanced materials. Successful students will learn skills in protein structure determination and biomaterial design, and will be at the forefront of materials research and development.

References:

1) **A. P. S. Brogan**, N. Heldman, J. P. Hallett, and A. M. Belcher. (2019) Thermally robust solvent-free biofluids of M13 bacteriophage engineered for high compatibility with anhydrous ionic liquids. *Chem. Commun*. 55, 10752-10755.
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Mechanoregulation of cell-matrix interactions in human intestinal organoid-based models of inflammatory bowel disease

Project ID: 2020_003

1st supervisor: Eileen Gentleman (Centre for Craniofacial and Regenerative Biology)
2nd supervisor: Joana Neves (Centre for Host-Microbiome Interactions)

Project type: Experimental

Project Overview:
Inflammatory bowel disease (IBD) can impact the matrix surrounding the gut epithelium, causing fibrosis and fistulae; however, it is unknown whether mechanical changes to the intestinal wall are a cause or consequence of inflammation. This project will establish human intestinal organoid (HIO)-based models of IBD which will allow us to use a combination of microrheology and microindentation to monitor how HIO modulate their local mechanical properties. This interdisciplinary approach will allow us to unravel how cell-mediated mechanical changes to the mesenchyme contribute to IBD-like phenotypes in the epithelium. Through this, we aim to reveal novel matrix-modulating targets that can be exploited therapeutically to treat IBD.

Project Aims and Description:
Cells and tissues are known to respond to mechanical cues during development, tissue maintenance and in disease. However, cells are not merely passive sensors of physical signals, but rather actively modify their local environment via both extracellular matrix (ECM) production and degradation (Blache 2020). We have shown that within 3D biomaterial polymer networks (hydrogels) that mimic the native ECM, cells can modulate the mechanical stiffness of their surroundings and that this impacts cellular behaviours (Ferreira, 2018). In this project, we will extend these observations to study inflammatory bowel disease (IBD), a serious inflammatory condition in which changes to the ECM of the intestinal wall can result in gut fibrosis or fistula formation. It is unknown whether ECM changes are a consequence of inflammation or contribute to IBD; however, we hypothesise that mechanical changes mediated by mesenchymal matrix secretion/degradation contribute to pathological-like phenotypes in the intestinal epithelium.

Intestinal organoids (HIO) are a well-established, accessible model of the intestine, containing both epithelial and mesenchymal cells, and can be formed

from human induced pluripotent stem cells. Our preliminary data suggest that co-culture of HIO with type 1 innate lymphoid cells, which accumulate in the inflamed intestines of IBD patients, can prompt peri-organoid ECM remodelling (Olszak 2014; Jowett, in revision). Here we will use HIO and modifiable PEG-based synthetic hydrogels to create 'IBD-in-a-dish' models and then use a combination of atomic force microscopy-based microindentation and multiple particle tracking microrheology (MPT) to understand how HIO modulate the mechanical properties of their peri-organoid space and how this impacts cellular phenotypes. MPT uses the Brownian motion of particles to provide *in situ* measurements of local peri-organoid degradation at the scale of a bead (< 1μm diameter). AFM allows for the mapping of cell-mediated softening and stiffening of the peri-organoid space on a larger scale (bead-modified probe, ~50 μm).

First, we will map the extent to which HIO mechanically modify their local environment. To determine if mechanical changes are sufficient to prompt inflammatory phenotypes, we will incorporate chemical strategies (non-cell-mediated) to soften/stiffen hydrogels, mimicking pathological stiffening (fibrosis) or degradation (fistulae). To determine whether specific secreted proteins mediate HIO's ability to respond to mechanical modifications, we will repeat experiments with targeted RNAi against specific secreted proteins or tethering of specific proteins to the hydrogel. This approach will determine if mechanical changes are sufficient to drive inflammatory-like phenotypes and whether they are mediated by specific secreted proteins maintained in the peri-organoid space.

References:

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Biosynthesis of 2D nanomaterials in bacteria

Project ID: 2020_004

1st supervisor: Mark Green (Department of Physics)
2nd supervisor: Roland Fleck (Centre for Ultrastructural Imaging)

Project type: Experimental

Project Overview:
Nanomaterial have found use in biological imaging and therapy, although they remain relatively hard to prepare in large amounts. Here, we will explore the synthesis of advanced materials, useful in cancer therapy and energy applications, using bacteria as the reaction medium, providing a cheap and efficient route utilizing biological processes rather than expensive high temperature chemical routes. Electron microscopy provides unrivaled 3D resolution for in situ characterization of nanoparticle synthesis at near atomic resolution. The project will provide new insight into nanoparticle synthesis together with innovation in the application of electron microscopy for soft materials research.

Project Aims and Description:
The use of colloidal nanoparticles, such as quantum dots, has become routine now in biology, notably in imaging and therapy. To a lesser degree, nanomaterials are now finding use in energy applications such as solar cells and battery technology as the synthesis techniques mature, yielding higher quality materials that can be routinely and reproducibly prepared. The high quality of the materials prepared by solution techniques does however come with a significant production cost that actually prohibits their utilization in real life applications. One method that circumvents this limitation is biosynthesis – where a natural biological process is exploited to yield nanomaterials as a by-product after the introduction of two metallic salts that could result in a remedial toxic impact. By carefully choosing the desired material, precursors, biological system and chemistry, we have shown we can prepare high quality materials that can be used in real life applications (such as light emitting devices and biological imaging), cheaply and effectively using simple biological systems.

In this project we have identified a biological process to yield solid state materials that have been used in cancer therapy and battery technology. Arsenic chalcogenides (As_2E_3, E = S, Te) are a relatively unexplored, yet

important group of materials with a wide range of biological and optoelectronic properties, although few sophisticated synthetic pathways exist beyond primitive melt technologies. We have, with collaborators at UCL, identified a bacteria species, *Desulfotomaculum auripigmentum,* which eliminates As_2S_3 when exposed to a simple arsenic salt and cysteine.

We will explore where in the bacteria the material is formed, how it is formed and the material composition, such as particle structures, crystal phase and optoelectronic properties such as band gap and charge carrier capabilities.

We will characterise the site of nanoparticle synthesis in 3D by electron tomography and 3D array tomography. Approaches which Fleck has previously used to study a twin-arginine translocation (Tat) system and how these proteins arrange themselves in the inner membrane of *Escherichia coli* to permit passage of Tat substrates, whilst maintaining membrane integrity. These combined approaches will support both high resolution ultrastructural study of membrane associated event and nanoparticle synthesis. When further combined with energy-dispersive X-ray spectroscopy (EDS) and scanning transmission electron microscopy (STEM) information pertaining to the atomic organisation of the nanoparticles themselves is revealed.

To further enhance the resolution and directly study membranes and the synthesis of nanoparticles. We will develop novel cryo electron microscopy (cryo FIB lamella for cryo electron tomography and the *in situ* study of biological membranes and their organisation) strategies for the direct observation and characterisation of the membranes involved in synthesis and the particles themselves. This builds on current studies using lyophilisation and vitrification to preserve tissues without chemical fixation or processing artefacts for STEM EDS and cryo tomography of complex cells and tissues.

With collaborators at the University of Hertfordshire, we will also explore biogenic As_2S_3 as an anti-cancer drug (previous work has shown arsenic chalcogenides are useful in tackling platinum drug-resistant tumours).

We will use the same bacteria to engineer the biosynthesis of As_2Te_3, a 2D topological semiconducting material with a near-IR band gap, by replacing cysteine with a tellurium salt. Such materials have a range of application in the semiconductor industry (IR-devices, optical switches, thermoelectric devices and solar cell technology) and have not been prepared by biosynthetic or chemical routes to date.

Few molecular routes exist to arsenic chalcogenides as the volatile precursors required do not lend themselves to thermolytic synthesis due to their extreme toxicity – biosynthesis is possibly one of the few routes to such nanomaterials as the chemistry involved is benign and well established. This project brings together the physics, biology and chemistry of these new materials.

References:

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Developing multiscale models to study molecular transport into tissues

Project ID: 2020_005

1st supervisor: Chris Lorenz (Department of Physics)
2nd supervisor: Martin Ulmschneider (Department of Chemistry)

Project type: Computational

Project Overview:
The goal of this project is to develop realistic 3D tissue models to allow simulation of molecular transport across scales. Tissues rely on the supply of a wide range of nutrients and metabolites from circulation to carry out their biological functions. To arrive at their final cellular destinations these molecules need to cross a variety of physiological barriers. At present, this process is poorly understood, chiefly due to the absence of multiscale *in silico* models that allow capturing perfusion, extravasation, and diffusive flux in tissues in its entirety. In this project centimetre-scale organs will be constructed by integrating atomic detail models of physiological barriers into micrometer-scale models of the vasculature and tissue fine-structure.

Project Aims and Description:
Molecular transport into tissues is vital for organs to carry out their functions and for drug delivery. Preclinical drug development and optimisation currently relies on animal models to determine target tissue exposure to lead compounds. However, these models often correlate poorly with clinical tissue exposure, as human physiology is substantially different from that of rodents, which are typically used. Furthermore, the prohibitive cost of animal models is limiting optimisation to a handful of compounds. What is needed are accurate *in silico* models of organs to reduce cost and allow screening of arbitrary pharmacophore chemistries. A fundamental challenge for these models is the wide range of spatial and temporal scales. For example, en route to its cellular target a 1.2Å diameter O_2 molecule, diffuses across many micrometers cytoplasm, plasma, and extracellular matrix, crossing a number of biological barriers with nanometer-scales.

This project will leverage the tremendous growth in computing power to create a multiscale model that captures organ level structures across three spatial scales: (i) biological barriers, such as plasma membranes, will be modelled at atomic detail on the scale of 1-500nm. Passive diffusion and active transport functions will be captured via molecular mechanics simulations, and

(ii) diffusive transport across plasma and extracellular matrix on the 500-5000nm scales will be captured using coarse-grained molecular descriptions, and (iii) macroscopic scale models that capture the vascular network and tissue fine-structures will be modelled using geometric and grid-scale techniques on the 5μm-50mm scales. Subscales (i-ii) will be seamlessly anchored into the micro-organ model (iii) using a branched root system design that allows information from a large number of subscale systems to feed simultaneously into the micro-organ. This approach leverages the rapid parallelisation of computing architectures, with each spatial scale summarising the topological and functional information of the immediate sublevel.

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2) Y Wang, E Gallagher, C Jorgensen, EP Troendle, D Hu, PC Searson, **MB Ulmschneider**. (2019) An experimentally validated approach to calculate the blood-brain barrier permeability of small molecules. *Sci. Rep*. 9, 1-11.
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Mapping the conformational landscape of intrinsically dynamic proteins across broad time scales

Project ID: 2020_006

1st supervisor: Argyris Politis (Department of Chemistry)
2nd supervisor: Manuel Mueller (Department of Chemistry)

Project type: Experimental/Computational

Project Overview:
This studentship aims to dissect the conformational dynamics underpinning function in intrinsically dynamic proteins. To do so, we will employ a unique combination of hydrogen deuterium exchange mass spectrometry (HDX-MS)—carried out in timescales spanning five orders of magnitude—with biochemical tools and advanced modelling. To showcase our method, we will use the challenging p53 protein comprising both folded and intrinsically disordered regions. The gain in mechanistic understanding will enable a step-change in monitoring the dynamics of such a complex nanomachine and provide a template to understand—at molecular level—other difficult to tackle biological systems.

Project Aims and Description:
Despite advances in structure determination, probing the conformations of highly dynamic proteins remains a challenge. Key difficulties include restrictions in protein size and lack of tools to probe protein dynamics. This impedes knowledge on functional protein states. HDX-MS offers a sensitive tool for interrogating protein dynamics via the exchange of hydrogen to deuterium. In combination with microfluidics, it allows rapid, sub-second, mixing of the reagents (fastHDX). To develop our method, we have selected the tumour suppressor p53, a 200kDa tetrameric transcription factor. p53 is mutated in half of cancers, and therefore intensely studied, yet the structure and dynamics of the biochemically active tetramer are not well understood. This is due to the juxtaposition of well-folded (DNA binding & tetramerization domains) and intrinsically disordered regulatory regions (40% of the protein), which has hampered structural characterisation and necessitates the development of new physical approaches.

Year 1: The student will be trained in expressing, purifying and refolding p53 (MM). Once the protein is produced, we will optimize conditions for achieving high-sequence coverage in HDX-MS (AP). Within this timeframe

they will carry out preliminary *fast*HDX using a capillary-based setup. This will establish the feasibility of our workflow and prompt subsequent experiments.

Year 2-3: The student will study how the conformational landscape is altered by functionally relevant interactions. They will carry out HDX in the presence of target DNA oligomers of varying length, randomized control DNA, and domains of p53 binding partners Mdm2 and p300. These will allow us to: (i) benchmark the HDX workflow given that the local binding sites are known, and (ii) link intrinsically disordered regions to functional transitions of p53. The student will carry out data-driven modeling (using structural restraints derived from HDX-MS experiments) to visualise functional states. During this period, they will also start writing the paper.

Year 4: The student will finalise the paper and write up their thesis.

We will establish a workflow to characterise the dynamics of intrinsically disordered proteins in time and space. Given the biomedical importance of p53 and the lack of conclusive structural data in its functional form, we expect this project to lead to a high impact publication. It will form the basis for a joint grant application to be submitted to EPSRC. Our approach can readily be adapted to study p53 regulation by post-translational modifications and other proteins embodying both folded and disordered domains – a common property of mammalian proteins.

References:

1) Martens C, Shekhar M, Lau A, Tajkorshid E, **Politis A*.** (2019) Integrating hydrogen-deuterium exchange mass spectrometry with molecular dynamics simulations to probe lipid-modulated conformational changes in membrane proteins. *Nat. Protoc.* 14, 3183-204.
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Multi-scale investigation of the conformations of musk odorants and their binding to human musk receptors

Project ID: 2020_007

1st supervisor: Maria Sanz (Department of Chemistry)
2nd supervisor: Franca Fraternali (Randall Centre for Cell & Molecular Biophysics)

Project type: Experimental/Computational

Project Overview:
Musk odorants are key compounds in perfumery due to their distinctive animal and sensual notes. However, insight on the determinants of musk smell has been hampered by the lack of structural information on receptors and on the musks themselves. This project will determine the structural elements associated to musk smell by characterising musk conformations and their interactions with human musk receptors through the combination of multiscale experimental and computational studies. Our results will provide crucial data for understanding musk identification and, more broadly, their pharmacological effects. Our data will unlock opportunities for rational design and development of new musks.

Project Aims and Description:
Musk odorants are widely used in the perfume industry as the base notes for fragrances, cosmetics and household products. There are four different classes of musks, but because of toxicity and bio-degradability issues, only macrocyclic and alicyclic musks are currently in use, and there is strong interest in the perfume industry to develop new musks. However, knowledge of the molecular determinants that lead to musk odour is poor. Two human musk receptors have been identified, but no crystal structures have been reported for them. No conformational studies have been published for macrocyclic and alicyclic musks up to date. In this project we will advance our understanding of the molecular interactions involved in musk smell by conducting a multiscale investigation on musks, musk receptors, and their interactions. We will use spectroscopic methods as well as static and dynamic modelling at different length-scales to determine the conformations of prototypical macrocyclic and alicyclic musks and model their interactions with human musk receptors. This data is essential to develop a molecular-level understanding of musk smell and will aid identifying musk interactions relevant for their pharmacological effects.

Macrocyclic and alicyclic musks are very flexible, which makes them impervious to conformational studies using traditional techniques. X-ray crystal structure analyses are not viable as the crystals show a great degree of disorder and non-clear diffraction patterns are obtained. NMR does not have sufficient resolution during the time of the experiments to distinguish between conformations. We will use rotational spectroscopy, a technique ideally suited to conformational studies because of its high resolution, which enables unequivocal identification of conformers simultaneously present in the sample. We will take advantage of our bespoke broadband rotational spectrometer at King's, which has been developed to investigate large molecules and is ideal to study the macrocyclic and alicyclic musks proposed here.

In parallel to the experimental investigation of musk conformations, we will develop suitable models for the human musk receptors OR5AN1 and OR1A1 using homology modeling and hybrid quantum mechanics/molecular mechanics methods. Subsequently, we will examine the docking efficiency of the musk conformations previously identified experimentally and identify common structural determinants in macrocyclic and alicyclic musks.
The overall goals for the PhD involve:

1. Experimental determination of the conformations of prototypical macrocyclic musks.
2. Modelling of the human musk receptors OR5AN1 and OR1A1.
3. Characterization of the binding of the different conformations of prototypical macrocyclic and alicyclic musks to the human musk receptors.

References:

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Mechanoregulation of cytotoxic T cell target cell killing

Project ID: 2020_008

1st supervisor: Katelyn Spillane (Department of Physics)
2nd supervisor: Robert Köchl (Department of Immunobiology)

Project type: Experimental

Project Overview:
Cytotoxic T cells form immune synapses with infected or transformed cells to instruct those cells to die. The process is selective, sensitive, and rapid and is initiated by piconewton-scale forces transmitted to receptor-antigen bonds. Whether mechanical noise from the environment dysregulates these interactions, or whether the immune synapse can insulate against large external forces, is not known. Here we will investigate how mechanical forces from the environment influence the mechanical and chemical signals in the immune synapse that enable cytotoxic T cells to recognise and destroy their targets.

Project Aims and Description:
Cytotoxic T cells are of central importance to the adaptive immune response and to cell-based anti-cancer immunotherapies because they are highly effective at killing infected or transformed cells. They become activated upon recognising antigenic peptide-major histocompatibility complexes (pMHC) on the surfaces of target cells. Recognition requires specific binding interactions between the T cell receptor (TCR) and pMHC and the transmission of piconewton-scale forces to TCR-pMHC bonds. These interactions initiate signalling cascades leading to reorganisation of the T cell actin cytoskeleton and formation of an immune synapse. Within the immune synapse, T cells secrete the protein perforin and a mixture of toxic proteases (a process called degranulation). Perforins form holes in the membranes of target cells, triggering a repair response that enables the proteases to enter into the target cell cytoplasm and induce apoptosis. Recently, it was discovered that T cells enhance this process by exerting nanonewton-scale forces against the target cell membrane to increase its tension and thereby enhance perforin activity, suggesting that mechanical forces may enhance the potency of chemical signals in the immune synapse [1].

Target cells can include cells within solid tumours, which tend to be very stiff due to enhanced cytoskeletal activity and a rigid extracellular matrix; and metastatic cells that have moved away from the tumour and tend to be softer

than their non-transformed counterparts. How cytotoxic T cells adapt to different mechanical stimuli to maintain their ability to kill their targets is unclear. For instance, T cells activate in response to a narrow range of piconewton-scale forces transmitted to TCR-pMHC bonds [2], but whether these interactions are dysregulated by changes in the target cell stiffness, or whether the immune synapse insulates them from external influences, is not known. Additionally, while the membrane tension of target cells reflects the rigidity of the extracellular matrix, whether T cells can adapt to these changes by modifying the nanonewton-scale forces they exert against the targets has not been investigated.

In this project, we will address these questions using biophysical assays to quantify forces from the piconewton-to-nanonewton range and high-resolution fluorescence imaging to visualise degranulation in the T cell immune synapse. We will combine DNA-based molecular tension sensors [4,5] with traction force microscopy to measure simultaneously the piconewton-scale forces transmitted to individual TCR-pMHC bonds and the nanonewton-scale forces that T cells exert to enhance perforin pore formation on the target cell. We will incorporate these force measurements with fluorescence imaging of degranulation to investigate how forces at different scales regulate chemical signalling in the T cell immune synapse.

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Time-correlated single photon-based lightsheet fluorescence lifetime imaging microscopy

Project ID: 2020_009

1st supervisor: Klaus Suhling (Department of Physics)
2nd supervisor: Maddy Parsons (Randall Centre for Cell & Molecular Biophysics)

Project type: Experimental

Project Overview:
In lightsheet microscopy, a thin slice of the sample is illuminated, and the image is observed at right angles with a camera. Fluorescence lifetime imaging (FLIM) can image complex dynamic processes, and to help us to understand life and disease on a molecular scale. FLIM is best done by assembling the image from individual photons - the most accurate and sensitive way of doing this. Conventional cameras can capture images well, but they cannot photon count in the way needed for lightsheet FLIM. The project will use a single-photon sensitive FLIM lightsheet microscope with a special photon counting camera. This unique instrument will be used to make movies of fluorescence under low light conditions in live cells in complex 3D environments to study diffusion of small fluorophores.

Project Aims and Description:
The aim is to optimise a delay line anode detector for a lightsheet microscope. It enables time-correlated single photon counting-based lightsheet Fluorescence Lifetime Imaging (FLIM) microscopy. Microscope alignment and optimisation of the optics and the detector read-out for lightsheet microscopy will be completed in 6 months. Development and optimisation of the data processing package will continue in conjunction with imaging of biological samples provided by academic collaborators during months 6 to 12. The first samples will only require the lifetime to be observed, but once this is demonstrated, samples with FRET pairs or biosensors will be imaged and will require the FRET efficiency to be quantified. Imaging large living biological samples with high resolution over extended periods of time in three dimensions is very hard using any other microscopy approach. Functional imaging in this context is even harder. Nevertheless, we aim to show that this is possible, and will illustrate the benefits of this lightsheet FLIM microscope through imaging selected biological examples such as cell spheroids, organoids and live ex-vivo tissue sections expressing fluorescent probes and biosensors. The resulting data will provide

novel means to interrogate cell function in large biological preparations and reveal new information regarding cell behaviour and signalling within complex environments.

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New tools for neurobiology: linking neurons and artificial cells

Project ID: 2020_010

1st supervisor: Mark Wallace (Department of Chemistry)
2nd supervisor: Juan Burrone (Centre for Developmental Neurobiology)

Project type: Experimental

Project Overview:
Brain-computer interfaces typically rely on invasive electrodes. A major flaw in these approaches is the mismatch between the practical number of electrodes, and the number of neurons. Recent advances in bottom-up synthetic biology suggest that artificial cells might provide an alternative route to create soft, biocompatible, brain interfaces that would circumvent current limitations. Here we will design basic interconnects between artificial cells and neuronal cells to create proof-of-principle sensors and actuators of neuronal function. This toolset will provide new routes to help understand the nanoscopic functional organization of neuronal networks.

Project Aims and Description:
Aim 1: A robust 'artificial synapse'. *We will create a direct synaptic junction between a Giant Unilamellar Veiscle and a neuron.* SynCAM is a brain-specific, immunoglobulin domain-based homophilic cell adhesion molecule in the synapse. We will reconstitute recombinantly-expressed SynCAM into Giant Unilamellar Vesicles (GUVs) to form artificial synapses with hippocampal neurons. Using methods established in our lab phase-transfer will be used to load GUVs with different cargos. We will use super-resolution imaging, confocal microscopy, and optical single channel recording to quantify this and subsequent processes.

Aim 2: Sensors. *We will explore two different routes to develop GUV-based sensors of local neuronal function.*
2.1: Indirect sensors of local action potential. Reconstituting fluorogenic voltage-sensitive domains (e.g. ArcLight) in GUVs will provide local measurements of neuron activity within the artificial synapse.
2.2: Indirect electrical sensing of calcium release. GCamp7 is a GFP-based indicator of calcium. Incorporated into neurons, this local fluorescence response will be detected in a GUV through the reconstitution of green light

sensitive opsins (e.g. eNpHR3.0). Opsin channel conduction will be measured in the GUV via micropipette aspiration.

Aim 3: Actuators. *We will create mechanisms to trigger an action potential based in response to local changes in artificial cell status.*
3.1: We will trigger local neurotransmitter release using artificial neurotransmitter vesicles. GUVs encapsulating neurotransmitter-loaded small unilamellar vesicles (SUVs) will be prepared. By using lipid-DNA anchors with defined melt-temperatures SUV fusion with the GUV membrane can be controlled Lowering the temperature below this threshold will caue cause artificial vesicle binding and fusion and neurotransmitter release into an artificial synapse formed between the GUV and a neuron.
3.2: Disulphide-locked alpha-hemolysin nanopores will also be used as a triggerable mechanism for localized neurotransmitter release.

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Integrated confocal Raman-fluorescence microscopy for intracellular protein and lipid imaging in neural stem cell cultures

Project ID: 2020_011

1st supervisor: Mads Bergholt (Centre for Craniofacial and Regenerative Biology)
2nd supervisor: Andrea Serio (Centre for Craniofacial and Regenerative Biology)

Project type: Experimental

Project Overview:
Lipids of the nervous system have major consequences for brain structure, function, behaviour and diseases. State-of-the-art fluorescence microscopy is specific for proteins but offers no insights into lipids. The goal of this PhD project is to drive forward a new paradigm of microscopy that enables fully correlative lipid and protein imaging. We will introduce a ground-breaking new confocal fluorescence microscope through novel integration with confocal Raman spectroscopy. We will develop a pipeline for correlative protein and lipid imaging from the cellular to tissue scale. We will finally apply the technique to characterise lipid composition, lipid rafts dynamics and time-dependent changes in elongating spinal cord axons and cortical axons.

Project Aims and Description:
Microscopy imaging techniques have allowed cell biologists to probe cell structure and function in previously unattainable details. These methodologies continue to evolve, with new improvements that allow tailoring the available techniques to a particular need and application. The majority of microscopy techniques in life science are based on exogenous fluorophores for protein imaging. These modalities, however, have shortcomings for imaging cell lipids. At present, scientists rely often on lipid composition data acquired largely by bulk systems (e.g. HPLC). Lipids, fatty acids and sterols are key components of plasma membranes and intracellular droplets, and are heterogeneously mixed in different membranes, both at bulk levels and with sub-domain spatial heterogeneity. In particular, in the nervous system these compositional changes have major consequences for brain structure, function and behaviour. For example, several studies have shown that lipid composition changes in neurons and other cells in the nervous systems occur -and have important consequences for- Alzheimer's Diseases, Parkinson's Disease, Multiple

Sclerosis and many other disorders. Nevertheless, very little is known about the role of lipids mainly due to lack of imaging capability.

Here we will develop a new imaging modality for simultaneous confocal imaging of protein and lipids based on a prototype developed within a collaboration between the supervisors. We will use a novel optical design scheme that combines visible excitation confocal Raman spectroscopy with a range of NIR excited fluorophores. This will allow us to perform fully correlative protein and lipid imaging simultaneously without Raman or fluorescence compromising each other. This project will take advantage of the comprehensive pipeline for imaging and spectral analysis already developed by the supervisors for correlative imaging [ACS Central Science 2017]. As a proof-of-principle of its application we will use this new technique to characterise the lipid composition of stem cell derived populations of human neurons and astrocytes in cultures, to obtain the first maps of lipid domains in neural cells with subcellular resolution. Building these maps will allow to study lipid heterogeneity and time-dependent changes occurring during axonal elongation and pathfinding, but also connected to the cellular maturation processes, and will both be an ideal application of the new technique and an important biological question with implication basic and translational biology.

Aims

The vision, which drives this proposal, is to develop a novel imaging platform that offers comprehensive imaging of proteins and lipids simultaneously. In order to archive this vision, the following research objectives have been formulated:

1. Development of the confocal Raman/fluorescence microscope for simultaneous protein and lipid imaging
2. Development of a computational pipeline and toolbox for imaging and spectral analysis
3. Characterise lipid composition, lipid rafts dynamics and time-dependent changes in elongating spinal cord axons and cortical axons, correlating it with cytoskeletal staining

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Exploring the mechanisms of action of dietary polyphenols in the vascular system across scales

Project ID: 2020_012

1st supervisor: Carla Molteni (Department of Physics)
2nd supervisor: Ana Rodriguez-Mateos (Division of Diabetes and Nutritional Sciences)

Project type: Computational/Experimental

Project Overview:
Cardiovascular disease is less common in premenopausal women, suggesting vascular benefits of estrogen. Clinical studies have highlighted that a diet rich in polyphenols present in berries can improve vascular function in women. The structure of such polyphenols has similarities to estrogen and they may therefore interact with estrogen receptors. We will explore whether and how selected phenolic metabolites, in which the ingested polyphenols are transformed after consumption, interact with estrogen receptors through innovative computer simulation methods to elucidate their potential mechanisms of action at the molecular level. This will complement in vitro and in vivo studies to elucidate how the molecular details translate across the cellular and macroscopic scales.

Project Aims and Description:
There is increasing interest in understanding how natural products, such as those derived from certain food, act on biological systems and processes, as such knowledge can have a tangible impact on health, as well as on the food, supplement and pharmaceutical industries.

Preliminary evidence suggests, for example, that dietary polyphenols from berries and red wine may exert cardiovascular health benefits in women via regulation of the estrogen receptor alpha (ERα), with which they may interact due to their chemical structure's similarity with estrogen. Dr Rodriguez-Mateos has recently identified more than 60 different polyphenols-derived metabolites in human plasma after berry consumption, some of which correlate with improvements in human vascular function.[1]

The goal of this PhD project is to investigate, through a multi-scale interdisciplinary approach, whether the effects of polyphenol-derived in vivo metabolites in vascular function are mediated via a mechanism involving ERα.

Building on Prof. Molteni's expertise in atomistic simulations of biomolecules including polyphenols [2,3], innovative computational protocols based on enhanced sampling methods (in particular metadynamics) will be developed to assess the ability of individual metabolites to bind to estrogen receptors and gain information on binding free energy affinities, mechanisms and paths, which cannot be obtained with conventional ligand-protein docking and molecular dynamics [4]. The anti-oxidant properties of the polyphenols will also be explored at the quantum mechanical level.

The computational work will complement in vitro and in vivo studies to elucidate how the molecular details uncovered in simulations translate across larger scales.

In Dr Rodriguez-Mateos' laboratory, to test whether ERα inhibitors can inhibit the effects of selected polyphenol metabolites, a physiologically relevant in vitro model of the vascular system (co-culture between endothelial cells and vascular smooth cells) will be used focusing on AKT, eNOS and PI3K phosphorylation, changes in eNOS protein and NO production. In collaboration with Dr Dr Paul Taylor (Reader in Women's Health at King's College London), studies in mice will allow characterization of the in vivo physiological effects of dietary polyphenols by employing state-of-the-art non-invasive ultrasound imaging of cardiovascular function.[5]

This interdisciplinary multi-scale approach will provide a unique set of complementary information to understand the beneficial effects of certain food on women's health.

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The impact of topography-induced local cytoskeletal rearrangement on metabolic cell requirements

Project ID: 2020_013

1st supervisor: Ciro Chiappini (Centre for Craniofacial and Regenerative Biology)
2nd supervisor: Andrea Serio (Centre for Craniofacial and Regenerative Biology)

Project type: Experimental

Project Overview:
Topographical cues are widely investigated as microenvironmental stimuli for stem cells differentiation in regenerative medicine. In particular, we have recently shown that high aspect ratio nanomaterials (nanoneedles) stimulate directly multiple elements of the cell, inducing local rearrangements of endocytic vesicles, cytoskeleton, and nuclear envelope. Yet, to date there is no systematic study focusing on how these dramatic rearrangements impact the organelle shuttling and distribution across cell compartments or how organelle dynamics are carried through these strongly altered networks. In this project we will focus on the effect of cytoskeletal local pinning, wrapping and sharp bending around nanoneedles on shuttling dynamics, biogenesis and function of mitochondria in neural progenitors, neurons and astrocytes.

Project Aims and Description:
Shuttling of mitochondria from the cell body to the cell periphery is a crucial requirement for maintaining metabolism in both neurons and astrocytes, and is a function that is targeted by several neurodegenerative conditions. Moreover, biogenesis and turnover mechanisms for mitochondria are intimately linked to their shuttling across cell compartments: most mitochondria need to be produced and disposed of within the cell body, but they are mainly required to maintain function within axons or astrocyte processes, as they need to regulate ATP/ADP ratio across the cells to match supply and demand. These functions are only possible if the network of cytoskeletal filaments on which they have to travel is correctly shaped and organized.

A broad range of high aspect-ratio topographies – including arrays of micro- and nano-pillars, nanowires and nanoneedles – can instruct neuronal differentiation of human stem cells, presumably by providing appropriate cues for axonal elongation. These same nanotopographies severely impact cell

architecture by altering nuclear shape and cytoskeletal arrangement. In particular, we have shown that nanoneedle arrays induce signaling clustering[1], deform the nuclear envelope[2], disrupt actin fibers[2] and manipulate axonal elongation (unpublished).

Despite this clear evidence of a strong interaction between nanoneedles and organelles that play a key role in intracellular transport, to date there is no systematic investigation of their impact on organelle trafficking in general, and specifically mitochondria shuttling.

The goal of this project is to better understand the dependency of mitochondrial shuttling on cytoskeletal arrangement, by first studying the impact of high aspect ratio nanotopographies on mitochondrial dynamics and then leveraging this phenomenon to rationally design a platform for reliable regulation of mitochondrial dynamics within the cells. Such platform would be a key tool to model the "form-function" relationship between the shape of cytoskeletal networks and ultimately the homeostasis of metabolism across neural cells.

To achieve these goals, we combine nanofabrication and biointerface design (Chiappini) with in vitro neuromodelling and live imaging (Serio) to pursue the following aims:

Year 1:

- Investigate how nanoneedles remodel the different cytoskeletal networks (actin, microtubili) in neurons and astrocytes as a function of maturation level.

Year 2:

- Study the role of these local alterations on the long-range shuttling of organelles from cell body to cell periphery
- Dissect the structure and dynamics of local cytoskeletal rearrangement as a function of physicochemical parameters of the nanotopography.

Year 3:

- Investigate the impact of reshaped cytoskeletal networks on biogenesis and turnover of mitochondria.
- Model the underlying principles regulating intracellular transport on nanotopographies.

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Creating dynamic functional membrane structures; the molecular basis of biomembranes

Project ID: 2020_014

1st supervisor: Paula Booth (Department of Chemistry)
2nd supervisor: Snezhana Oliferenko (Randall Centre for Cell & Molecular Biophysics)

Project type: Experimental

Project Overview:
Biological systems organise themselves with efficiency and precision. We are far from a complete understanding of this natural self-assembly, which in turn limits our ability to mimic biological construction in tuneable synthetic systems. Natural membranes are formed from only two core components, proteins and lipids, but they conceal a functional sophistication that cannot be mimicked in artificial systems. We want to understand how this collective emergent behavior of membranes arises and exploit this in artificial cells. These goals will be achieved by integrating biophysics, chemistry and synthetic biology approaches on individual molecules through supramolecular chemistry to complex biological systems.

Project Aims and Description:
How life originates from simple chemical precursors is a fundamental unanswered question. The membranes that underpin cellular life have inspired a generation of smart materials, but these materials fail to attain the functional complexity of natural membranes. This project focusses on how proteins and lipids co-assemble to form intricate biomembranes capable of a plethora of highly regulated functions.

Membranes are complex, elastic entities. Studies over different time and length scales, from specific protein-lipid interactions, through individual membranes, to cells are required to address their dynamic supramolecular arrangements. The overall mechanics of membranes are critical and directly influence the function of constituent membrane proteins. In Nature, lipid and protein molecules of widely varying individual structures give rise to membranes with subtle differences in mechanical properties. The project aims to investigate 1. the molecular origins of different naturally occurring membrane mechanics and bending rigidities and how they influence membrane function, and 2. mimic and manipulate such self-assembly in synthetic systems to create bespoke artificial

membranes. We propose to combine our complementary expertise in membrane biophysics and proteins (Booth lab) with cell biology and lipid metabolism (Oliferenko lab) to tackle these aims.

We will exploit a recently identified naturally occurring unusual lipid composition. The related fission yeasts *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus* show differences in fundamental membrane-centered processes such as nuclear membrane remodeling. We recently discovered that these sister organisms exhibit different membrane lipid composition; *S. pombe* generates 'conventional' membranes where both phospholipid fatty acyl tails are approximately 18-carbon long. In contrast, *S. japonicus* synthesizes unusual 'asymmetric' phospholipids where two tails differ in length by 6-8 carbons. This results in stiffer, more phase separated bilayers.

A specialised analysis technique will be employed to determine bending rigidity by quantifying thermally-induced fluctuations of giant unilamellar vesicle membranes using confocal microscopy under phase contrast mode and a high speed camera. This will allow us to determine how the unusual asymmetric short chain lipids alter rigidity. Membranes made from cell extracts as well as synthetic lipid mixtures will be investigated. Moreover, the influence of increasing amounts of membrane proteins on the rigidities of membranes composed of different lipids will be assessed. We have identified single transmembrane helix families that have significantly different transmembrane helix lengths in the two yeasts. The work will enable us to expand the repertoire of synthetic membranes used as building blocks for artificial cells, and exploit mechanics to manipulate their function.

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