2023 SoCoBio DTP Annual Conference

Book of Abstracts

Second-year student talks (2021 cohort)

Susmita Aown, University of Sussex

Host plant relationships of potential insect vectors of *Xylella fastidiosa*

Xylella fastidiosa is a bacterial pathogen that causes disease symptoms in plants, such as leaf scorch and plant dieback. The outbreak of X. fastidiosa in Italy has affected 80% of all olive plantations and incurred a loss of €6.3 billion a year. X. fastidiosa has spread globally through transport of infected plant material. Consequently, there is a high chance that X. fastidiosa could enter the UK from continental Europe in the future. Philaenus spumarius is the main insect vector of X. fastidiosa locally in agricultural habitats in southern Europe. The X. fastidiosa bacterium is transmitted from infected plants to healthy plants by P. spumarius feeding. My research is focussed on understanding the feeding behaviour of P. spumarius, specifically the host plant preferences of the insect in the UK.

Austeja Bakulaite, University of Portsmouth

Development of novel small molecule inhibitors of Tyro3 receptor tyrosine kinas

Tyro3 is a member of TAM (Tyro3, Axl and Mertk) family, a subfamily of receptor tyrosine kinases. TAMs are involved in promotion of cell proliferation, survival, migration, tumour angiogenesis and suppression of anti-tumour immunity. Tyro3 is overexpressed in multiple different tumours and it is associated with poor patient prognosis. This makes Tyro3 an attractive target in anti-cancer therapeutics. At the moment, there are no clinically approved selective Tyro3 inhibitors. Previous work from our group using molecular docking analysis has identified a number of small molecules which virtually occupy the kinase active site pocket of Tyro3. Currently, we are working to test these hits as potential Tyro3 kinase inhibitors using both computer-based and experimental models. We have conducted further searches of a compound library which has yielded hundreds of new molecules similar in size and structure to one of the previously identified hit molecules. The top hits in the docking analysis are subsequently being tested in vitro in a cellular assay measuring their effects on human cancer cell viability, using different cell lines with distinct TAM expression profiles. This work should yield a number of novel small molecules that are selective inhibitors of Tyro3, and which could subsequently be further studied for development as potential cancer therapeutic agents.

Alice Clark, University of Sussex

Visual surveys vs eDNA metabarcoding to monitor biodiversity in Sussex post trawling ban

Kelp forests are ecosystem engineers, acting as nursery grounds for fish, playing a role in storm mitigation and sequestering CO2 from the atmosphere. Until the 1980s, kelp was abundant along the Sussex Coast, but years of fish trawling and destructive storms have led to the loss of these dense kelp beds. In March 2021, a trawling byelaw was introduced by the Sussex IFCA (Inshore Fisheries and Conservation Authority), banning fish trawling along 300 km2 of the Sussex Coast. This byelaw, and the resulting expected ecosystem recovery, offer an opportunity for the exploration of new techniques in marine biomonitoring. Here we use Baited Remote Underwater Video (BRUVs) and environmental DNA (eDNA) to monitor the biodiversity of Sussex Bay at 28 sites, inside and outside the trawling exclusion zone. After two years of monitoring, we have found a shift in community composition driven by an increase in macroalgae coverage in 2022. In addition, our findings demonstrate that eDNA is a much more powerful biomonitoring tool for surveying marine ecosystems than BRUVs, detecting almost 3 times as many species.

Matthew Davis-Lunn, University of Southampton

Promoting neurite outgrowth through the integrin adhesion complex

Spinal cord injury can result in severe damage to the axonal fibres that send commands from brain to body. Recovery is dependent upon regeneration of axons through the injury site, which is largely unsuccessful due to a combination of an inhibitory extracellular environment, and an intrinsic lack of regenerative ability. Current regenerative therapies are extremely limited, with most treatments only able to minimise damage to the central nervous system. Gene therapy by delivery of a specific integrin receptor has emerged as a potential regenerative therapy, by enabling axons to adhere to, and grow through, lesioned extracellular matrix (ECM). The integrin receptor binds the ECM, enabling construction of intracellular protein complexes that mediate downstream signalling. Our project will target the adhesion complex to identify additional mechanisms promoting neurite outgrowth in vitro, with the aim of developing a cotherapy to enhance efficacy of integrin-mediated neuroregeneration.

Fiona Dresel, University of Kent

Investigating metabolic dysfunction as a driver of the Motor Neuron Disease Amytrophic Lateral Sclerosis

The neurodegenerative disorder Amyotrophic lateral Sclerosis (ALS) leads to a progressive loss of motor neurons controlling voluntary muscles ultimately leading to paralysis and death usually through respiratory paralysis. Recent findings suggest that metabolic defects in combination with lower abundance and diversity of the bacterial profile in ALS patients play an important role in the onset and progression of ALS. Dietary compounds are metabolised by the gut microbiota yielding metabolites altering the metabolic status of the host influencing host health together with mutations in the proteins Superoxide dismutase 1 (Sod1) and Calcineurin this can be linked to familial and sporadic forms of ALS. Hallmarks of the disease are calcium dysregulation in motor neurons and defects in energy metabolism. However, the exact interplay between energy metabolism and calcium signalling and the effect of Sod1 and calcineurin defects are yet to be characterised.
We developed high throughput yeast and C. elegans models of ALS enabling us to probe the metabolic nature of Sod1 toxicity. We show that Sod1 mutations interfere with calcium and energy homeostasis in the absence of certain metabolites and genes encoding amino acid, metal ion and other transporters located on the vacuole membrane. Our results highlight some new interactions between Sod1 and calcium signalling which may improve our understanding of the metabolic dysfunction of ALS.

Johanna Fish, University of Southampton

VHL and UPS: delivering PROTACtion

Dysregulation of the ubiquitin proteasomal system (UPS) can cause accumulation of damaged or misfolded proteins, and disruption of other signalling pathways, leading to a wide array of maladies. The abundance of E3 ligase signalling enzymes in this system provides a new modality to assist protein degradation through catalytic UPS ‘hijackers’, namely, PROteolysis-TArgeting Chimeras (PROTACs). These molecules comprise three parts: a protein of interest (POI) warhead and an E3 ligase targeting warhead connected by a linker to facilitate degradation by the UPS. Despite the benefits of protein degradation over reversible inhibition, they suffer from poor pharmacological properties. Thus, modifications to improve cell permeability and bioavailability is pertinent for their therapeutic viability. We are developing the E3 ligase targeting moiety of the PROTAC for drug optimisation, which recruits the von Hippel-Lindau (VHL) tumour suppressor protein, a component of an E3 ubiquitin ligase complex. Through structure-based design and in silico studies, we aim to identify new synthetic ligands of VHL to improve the physicochemical properties of their resulting PROTACs. We have developed scalable syntheses for these scaffolds, incorporating our first generation of modifications. These will undergo evaluation using a range of biophysical methods

and biological assays. The results will direct our next round of modifications and later we will evaluate the properties in the overall PROTAC construct. These molecules, applied across a wider range of diseases, could be significant for drug discovery and improving patient outcomes.

Amanda Gilbert, University of Southampton

Manipulation of Oxygen to Induce a Model of Liver Zonation

The liver is involved in diverse, often contrasting physiological roles. These include: nutrient storage, detoxification of substances, gluconeogenesis and glucose metabolism. To maintain efficiency, opposing functions are spatially separated through zonation of the key functional cells, hepatocytes. Hepatic zonation is split into three: the periportal zone, closest to the portal vein and hepatic artery, pericentral zone, next to the pericentral vein and the mid-lobular zone, between the two. Zonation is predominantly driven by availability of oxygen. Oxygenated blood flows through periportal towards pericentral, resulting in an oxygen gradient of 8.5% to 4% respectively. This drives differential gene expression. Compromised liver function by disease or toxic substances is detrimental to morbidity and mortality with liver disease being the 3rd leading cause of premature death in the UK. Furthermore, the liver's role in the drug metabolism, results in damage due to adverse drug reactions. Currently the only treatment for end stage liver disease is transplantation, but there is a donor shortage. Production of accurate, high fidelity in vitro models provides opportunities to develop treatments for liver disease, generate material for transplantation and enable quicker, cheaper screening of potential drugs. Current models overlook the zonated structure of the liver, do not generate fully mature hepatocytes and cannot be used for long-term studies. By recapitulating the zonated liver we will better understand mature hepatocytes and their functions. This project aims to manipulate oxygen availability, to create a high fidelity model of the zonated liver.

Erick Gomes Oliveira, NIAB; University of Southampton

Manipulation of chloroplast density to enhance photosynthesis and nutritional value of tomato

As global population growth continues to put pressure on existing agricultural systems, improving photosynthetic efficiency becomes ever more important to achieve sustainable food security. The ability of crop plants to convert light energy into chemical energy to sustain plant growth is achieved through chloroplasts. In fruits, this is particularly interesting as the conversion from chloroplast to chromoplast during the ripening process is associated with chlorophyll degradation and the accumulation of carotenoids in the chromoplasts, leading to the production of the secondary metabolites associated with the fruit's organoleptic qualities. Therefore, the genetic manipulation of fruit chloroplasts could simultaneously improve both fruit size and quality traits. The overall aim of this study is to manipulate chloroplast development and density in fruit to produce plants with higher

pigment content, enhanced fruit photosynthetic performance, and increased yield. To achieve that, we have identified target proteins for the manipulation of chloroplast development. Overexpression of a MADS-box transcription factor from Birch in Tobacco was shown to enhance the growth and division rate of chloroplasts. Furthermore, various transcription factors expressed in Arabidopsis have been shown to increase chlorophyll content and chloroplast number. To date, I have used Golden Gate cloning to generate BpMADS overexpression constructs under the control of early and ripening fruit-specific promoters. We are currently generating transgenic tomato plants (Solanum lycopersicum) with altered levels of the targeted proteins and will evaluate the impact of chloroplast number on fruit organic compound sequestration, nutritional value, and postharvest quality. The technology proposed in this project represents an important milestone in the genetic manipulation of fruit photosynthesis and could be utilised in other fruit crops.

Matthew Irwin, University of Southampton

Utilisation of bacteriophage-based bioengineering for the enhancement of wastewater treatment efficiency

Wastewater treatment plants (WWTPs) face increasingly stringent nutrient discharge standards to protect environmental water bodies and human health. Poly-phosphate accumulating organisms (PAOs), are microbes that mediate enhanced biological phosphorus removal (EBPR). PAO however are often outcompeted glycogen accumulating organisms (GAOs), limiting phosphorus removal. Previous research has identified a link between GAO abundance and elevated wastewater temperature. Current control mechanisms for limiting GAOs currently require physical or chemical process alterations. However, these processes have been shown to limit the performance of other aspects of biological nutrient removal (BNR) and increase nitrogen-based greenhouse gas (GHG) emissions. With the advent of rising global temperatures more regions globally are expected to operate with elevated levels of GAO competition. In addition, some WWTPs already operate at elevated temperatures due to geographical location, seasonal temperature variations and/or heat-treated trade wastewater influent.

Noviann Mclean, University of Kent

Using C. elegans to understand appetite regulation and its associated metabolic traits

Appetite and satiety responses are key to the health and survival of an organism. These food-related behaviours require complex integration of external sensory and internal cues and dysregulation of these can result in metabolic disorders including obesity and its related co-morbidities. Previous studies have shown that in the nematode worm, C. elegans, a neuronal transcription factor called SKN-1B regulates appetite and satiety behaviours as well as controlling

metabolic homeostasis (Tataridas-Pallas et al., 2021 PLoS Genetics). SKN-1B is the worm ortholog to the mammalian NF-E2 related transcription factors (Nrfs) and acts in two ASI chemosensory neurons. Our aim is to understand how neuronal SKN-1B/Nrfs act to control appetite and body size, and to define the changes in gene expression in response to skn-1b mutation and feeding and how these impact metabolism and physiology. Here, we have used a combination of genetics and microscopy to examine: 1) the effect of SKN-1B on energy stores such as fat and how this is affected by ageing; 2) the effect of skn-1b mutation on mitochondrial health using oxygen consumption as a readout (both under normal conditions and in response to respiratory stress); and 3) the neuronal signals that SKN-1B requires to communicate dietary signals to key metabolic tissues in the worm and control behaviour and metabolism. Our work could give insight into the role of neuronally expressed Nrfs in behaviours related to appetite regulation in mammals.

Henry Nvenankeng, University of Southampton

Nicotinic acetylcholine receptor EAT-2, a novel pharmacophore for the mitigation of parasitic nematode infections

Parasitic nematodes infect humans, animals and plants causing diseases and severe crop losses. In agriculture, losses due to plant parasitic nematodes have been estimated to reach about $US157 billion annually. Synthetic chemical pesticides have been widely used and proven to be effective in disrupting parasitism and maintaining crop health. However, concerns about their extensive off-target effects on beneficial organisms and several eco-toxic impacts drive the need for target selective chemicals that are less persistent in the environment. In this research, we explore a unique nicotinic acetylcholine receptor (nAChR) EAT-2, in the model organism Caenorhabditis elegans as a novel drug target. The EAT-2 receptor is expressed in the nematode pharynx and is a major determinant for feeding. Its uniqueness among nAChRs and presence in several economically important nematode species makes it a potential target for compounds that could disrupt its physiological function and eventually resulting in death of parasitic worms (Choudhary et al., 2020). Using a full organism bioassay, we show that the EAT-2 receptor in C. elegans can be pharmacologically targeted to disrupt feeding. This was evidenced by measuring phenotypic behaviours like pharyngeal pumping and motility in the wildtype (N2) and mutant strains eat-2(ad465) and lev-1(x427). From this assay the EAT-2 receptor was activated by the agonist nicotine, which caused a hypercontraction of the nematode pharynx resulting in pharyngeal pump inhibition. Using a medium throughput screening platform, we have screened a library of compounds to identify any that show EAT-2 activity. Identifying putative compounds could improve the chances of finding compounds that are target selective and/or specific.

Thomas Paige, University of Kent

Linking structure to function in the cryptic DedA family of integral membrane proteins

The DedA family are integral membrane proteins that are found throughout eukaryotes, prokaryotes, and archaea. In bacteria disruption of DedA function leads to phenotypes including antimicrobial sensitivity, temperature sensitivity, cell division defects and pH sensitivity. The definitive function however of the DedA family appear cryptic and varied between homologues. These varied phenotypes mean that DedA proteins are targets for inhibition either as antibiotic targets or to sensitise cells to other drugs or extracellular conditions. For that to become apparent, we need to study their structure/mechanism and link it to function to truly understand how they are involved in so many varied phenotypes. *E. coli* has 8 DedA homologues that have been widely studied, with the most characterised homologue YqjA being the model bacterial DedA in the few biochemical/structural studies published. Here we use YqjA as our structural model to investigate its function, mechanistically important amino acids, oligomeric state, associated lipids and complete 3D structure. We will use biochemical/biophysical techniques such as chemical crosslinking to analyse oligomeric state, thin layer chromatography for lipid analysis as well as a GFP thermal stability (GFP-TS) assay to measure the melting temperature under varying conditions and with potential substrates. The DedA family's importance is clear from their associated phenotypes, but their function and structure remain cryptic. This study will utilise assays to gain data on structure/oligomeric state, substrate specificity and how lipid interactions can affect both structure and substrate binding. This combination of data will allow us to convincingly link DedA structure to function.

Sophie Powell, University of Southampton

Investigating the role of PURA in neurodevelopment using CRISPR/Cas9 saturation genome editing

PURA is a small, extremely evolutionarily well-conserved gene which encodes a single-stranded nucleic acid-binding protein, Purα. In 2014, whole-exome sequencing studies revealed that de novo heterozygous dominant mutations in PURA are responsible for a severe neurodevelopmental disorder, now known as PURA Syndrome. PURA Syndrome is characterised by mild-to-moderate developmental delay with moderate-to-severe intellectual disability alongside a number of other symptoms that give a heterogenous patient population, including seizures, hypotonia and feeding difficulties. Clinical intervention currently involves management of these symptoms, as there is no known disease-modifying therapy for PURA Syndrome. Causative mutations can occur almost anywhere in PURA, but the relationship between the specific mutation and the presence and severity of patient symptoms remains unclear, limiting inferences that can be made about patient

diagnosis, prognosis and the scope of therapy design. This project will initially identify PURA variants-of-interest using saturation genome editing, a novel technique that utilises homology-directed repair CRISPR/Cas to simultaneously introduce and assay every possible single-nucleotide variant in each exon of a given gene. The variants will then be precisely recreated in Xenopus tropicalis frogs and characterised in terms of gene expression and localisation, brain development as well as behaviour, early locomotion and working memory phenotypes. By combining in vitro and in vivo techniques, this research aims to characterise the impact of individual PURA variants on neurodevelopment and further our understanding of how these variants contribute to the pathogenesis of PURA Syndrome.

Daniela Rothschild-Rodriguez, University of Southampton

Phage isolation for gut-modulation of Klebsiella pneumoniae: an IBD-associated pathogen

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a recurring an auto-inflammatory gut condition that causes lasting tissue damage. The presence of certain pathobionts in the gut microbiota, which influence abnormal immune responses, has been linked to the exacerbation of IBD. One of these key pathobionts is Klebsiella pneumoniae, specifically the clade ST323. K. pneumoniae is an "ESKAPE" pathogen, known for its high virulence and antibiotic resistant profile. It has been identified by the World Health Organisation as a critical pathogen requiring new antimicrobials. In our research, we have isolated over 50 phages against clinical strains of K. pneumoniae, seven of which target the IBD-associated ST323 strains. Our goal is to test the efficacy of these phages using the Caenorhabditis elegans nematodes as an in vivo model. Specifically, we aim to evaluate whether these phages could be used for A) phage therapy in standard clinical K. pneumoniae infections and B) clearing K. pneumoniae gut colonisation.

Molly Rutt, University of Southampton

Recurrent pregnancy loss and endometrial extracellular vesicles

Tragically, up to 3% of those attempting to conceive will experience recurrent pregnancy loss, clinically defined as three or more consecutive losses. While approximately half of early, first trimester losses are attributed to blastocyst genetic abnormalities, a large proportion remain unexplained, and limited medical investigation exists in regard to prediction, treatment or prevention of pregnancy loss. It is hypothesised up to 75% of these losses may be attributed to issues with embryo implantation, a process reliant on the concurrence of a viable blastocyst and a receptive womb. As such, the receptivity of the endometrium, the womb lining and surface into which the embryo implants, is a vital metric by which to predict implantation success.The endometrium contains a network of glands, which secrete signals to the embryo and endometrial lumen. One

such signal is extracellular vesicles (EVs). Using electron microscopy we have shown human endometrial glands secrete EVs from the tips of their epithelial cell microvilli. We then use flow cytometry to detect and characterise EVs in endometrial fluid from the womb lumen, where they range from 100-1000 nm in size Having isolated EVs from endometrial fluid we have shown that they contain populations of microRNAs, which may be delivered to target cells of the embryo and reproductive tract. As signalling molecules, these microRNAs can drive changes to cellular function or phenotype. As such, endometrial gland-derived EVs and their contents may be essential biomarkers for endometrial receptivity and as such, pregnancy success.

Annabelle Somers, University of Southampton

Parsnips aren't just for Christmas: Exploring Solutions for Micronutrient Insecurity in the UK

The United Kingdom is the fifth largest global economy, with a GDP of $2.8 trillion and the 15th highest Human Development Index ranking in the world. Despite this, between 2017 and 2019 the FAO categorised 0.9 million people in the United Kingdom as severely food insecure, surpassing all other European countries, and making the United Kingdom responsible for a tenth of all severely food insecure people in Europe. Improving micronutrient security requires micronutrient dense foods to be accessible, safe, and available for all people at all times. One crop that could fit this purpose is *Pastinaca sativa*, commonly known as parsnip. Parsnips are cheap, micronutrient dense, and substitutable for other starchy vegetables to become a main constituent of meals, providing calories, taste, and texture. They are produced domestically and store well beyond the cropping season, suggesting that they could provide a reliable year round source of nutrients. My research explores how variation from "farm to fork" affects parsnip micronutrient content, including the effects of crop variety, cooking, and storage. In addition, the place for parsnips in the wider food system will be assessed, by evaluation of the micronutrient content of meals in schools, care homes, and hospitals, and hypothesising how this would be altered by inclusion of more parsnip. Finally, future scenarios for the UK will be modelled, to explore how changes in parsnip production and consumption in future may fit with climate, health and economic scenarios.

Richard Stack, University of Kent

Benefits of creating a mock community in the characterisation of reproductive microbiomes

The relationships between the microbiome of the male reproductive tract, semen quality and fertility metrics, remain poorly defined. Clustering of community state types is inconsistent between research groups and a clear characterisation of dysbiosis is elusive. Whilst high-throughput sequencing technologies have allowed for resolution of previously

unculturable microbes, the sensitivity of these techniques create multiple opportunities for bias. In an attempt to test our sequencing pipeline for bias, we created a mock microbial community. By combining axenic cultures isolated from boar semen in equivalent ratios, we aimed to create a dataset that, whilst idealised, carried relevance to the natural microbial community in terms of phylogenetic representation. Individual mock community members provided data on the effectiveness of lysis and DNA extraction, whilst relative abundances of taxa at the end of our workflow allowed for identification of false positives and negatives.
Filtering of taxa is commonly undertaken by removing singletons, i.e. taxa that appear once across the dataset. This approach does not take sample number or depth into consideration, and can skew community relationships. We used mock community data to inform our comparison of both within (alpha) and between (beta) metagenome diversity, by using it to define the threshold for removal of false negatives across all samples. The optimisation of sequencing workflows through use of a mock community, imparts greater confidence in the characterisation of reproductive microbiota, and the promise of revealing their relationship to fertility.

Bree Streather, University of Kent

Using a novel recombinant vesicle nucleating peptide to produce protein in anaerobically grown *E. coli*

Eastwood et al. (2022) (DOI: https://doi.org/10.1016/j.crmeth.2023.100396) describe an innovative peptide tagging based technology that allows the production of diverse recombinant proteins in *E. coli* that are subsequently packaged into membrane-bound vesicles and exported into the media. This has many advantages including simplified downstream processing and storage. It has also been shown to significantly increase yield of particularly challenging or insoluble proteins. The work described in this talk attempts to use this technology in anaerobically grown *E. coli* with the ultimate aim of producing proteins that require reducing conditions i.e. oxygen-poor. Renewed optimisation of the system was required for it to function in this unfavourable environment.

Jack Stubbs, University of Southampton

From structure to time-resolved serial crystallography

Around 94% of structures currently in the PDB were collected at 100 K (cryogenic), however living systems function at physiological temperature and pressure, therefore we must collect data at room temperature. The advent of protein structure prediction models such as AlphaFold and RosettaFold, highlights further the need to transition into the field of time-resolved dynamic structural biology. No longer is it enough to know just the structure-function relationships, but also the dynamics.The protein target we utilise for time-resolved studies is Pdx1, an enzyme that synthesises pyridoxal 5’-phosphate (PLP), an active form of vitamin B6, by reacting glyceraldehyde 3-phosphate

and ribose 5-phosphate with ammonia from the corresponding Pdx2. Intermediate structures were previously determined, but these are end-points and with the aid of time-resolved serial crystallography, we now aim to observe fascinating enzyme transition states in crystals.In order to determine time-resolved structures of macromolecules engaged in catalysis within a crystal, we must transition from generating a large single crystal to thousands of microcrystals. We have now optimised the crystallisation of Pdx1 to produce large amounts of microcrystals, and performed the initial proof of principle serial experiments at both synchrotrons and XFELs.

Lucy Sutton, University of Southampton

Listeria monocytogenes biofilms in the fresh food supply chain

Salads and herbs are essential for a healthy diet but during their processing and packaging may be exposed to environmental contamination from foodborne pathogens. Ready-to-eat (RTE) produce is not cooked before consumption which increases the risk of foodborne disease outbreaks. Of particular concern is *Listeria monocytogenes*, which has a low infection rate but the highest mortality rate of foodborne pathogens. It is ubiquitous in nature and can grow and survive across a wide range of temperatures including refrigeration temperature (~4oC) – common to the fresh food supply chain. *L. monocytogenes* has been shown to enter the viable but non-culturable (VBNC) state due to stress from disinfectants, such as chlorine and peracetic acid. The VBNC state renders potentially infectious bacteria undetectable using standard culture recovery methods, and produce deemed safe may be harbouring foodborne pathogens. The data presented here show that there is a 3-log reduction in cell viability in 7-day *L. monocytogenes* Scott A biofilms grown at 4oC after 100 ppm chlorine treatment. Whereas the same biofilm treated with 50 ppm chlorine shows a less than 1-log reduction in cell viability – highlighting the necessity to treat biofilms with appropriate concentrations of disinfectant. Despite a successful decrease in cell viability, further analysis is needed to determine whether the cells are dead or VBNC. These results show promising advances in the treatment of listeria biofilms in the food processing environment and further work using Raman spectroscopy will be carried out to develop biomarkers of the VBNC state.

Kaya Taylor, University of Sussex

Exploiting *Mycobacterium tuberculosis* biofilm-derived phenotypes for transformative novel drug discovery

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB). TB is still a worldwide issue and is the leading cause of death from a bacterial infectious disease (WHO., 2021). The 2021 WHO tuberculosis report estimates that over 1.4 million people died in 2020 from active TB disease with the regions of South-East Asia, Africa and the Western Pacific having the highest proportion of cases (WHO., 2021). As a disease, TB can be very difficult to cure. This coupled with

the emergence of antibiotic resistant strains means that current drug regimens are lengthy and require a combination of anti-microbial drugs with toxic side effects. Therefore, novel drug discovery is fundamental to eradicating this disease. *M. tb* has been observed to grow as aggregated clumps or clusters of bacilli both *in vitro* and *in vivo,* as a single organism biofilm. This aggregated biofilm-like growth causes important changes to the phenotype of the bacilli, leading to phenotypic heterogeneity within the aggregates. *In vitro* modelling of *M. tb* biofilms show enhanced survival to antimicrobial drugs. Therefore, identifying and characterising these drug-tolerant *M. tb* populations may help to advance novel treatment-shortening drug regimens for TB or identify new drug targets.

Jamie Thomas, University of Southampton

Investigating the role of 5-Hydroxytryptamine receptor subtypes in the modulation of cell-specific immune function

5-Hydroxytryptamine (5-HT), also known as serotonin or 3-(2-aminoethyl)-1H-indol-5-ol, is a phylogenetically conserved biogenic monoamine that can act as a neurotransmitter, hormone, and mitogen, in various locations throughout the body (e.g., nervous system, cardiovascular system, immune system, etc). 5-HT signalling is primarily mediated by the family of 5-HT receptors (1-7), which comprise 15 known mammalian subtypes, that facilitate the activation of numerous intracellular signalling pathways associated with a wide range of biological activities in different cell types. Immunological studies show that almost all immune cells express one or more 5-HT receptor subtypes and many immunological function have been attributed to 5-HT signalling, via specific 5-HT receptor subtypes in different immune cells - highlighting the essential role that 5-HT has within the immune system. To date, the full extent of the expression and influence that different 5-HT receptor subtypes have on different immune cells remain relatively unknown. In this project, we aim to utilise a range of molecular, biochemical and cellular techniques to 1) investigate the expression of 5-HT receptor subtypes in a variety of immune cells, 2) characterise the immunomodulatory functions associated with 5-HT signalling, and 3) identify if these functions can be manipulated using known 5-HT receptor agonists/antagonists. This will develop our understanding of both the function of 5-HT and its roles within the immune system, which in turn could shed new light on our understanding of the immune system in general, alongside how it interacts with other organic systems (e.g., neuroimmune axis), under physiological conditions and in diseased states.

Konstantinos Tornesakis, University of Portsmouth

Evaluation of thermostability and kinetic stability of HotPETase, a thermotolerant PET hydrolase, and its predecessors in the directed evolution design process

Increased enzyme stability and effective catalysis at high temperatures are important features for biotechnological applications. The enzymatic recycling of PET plastic is particularly challenging,

degrading the polymer to its monomers. The enzyme IsPETase achieves this through a Serine-Histidine-Aspartic catalytic triad at ambient temperatures. Through directed evolution, a thermotolerant variant, named HotPETase, was created. This project aims at characterizing the thermostability and catalytic activity of those two enzymes and their intermediates in the evolutionary process by combining computational and experimental techniques. The PET degradation rates of all enzymes were investigated with time course assays, revealing different kinetic stabilities. Protein unfolding was studied with differential scanning calorimetry, while enzyme-PET binding was tested with isothermal titration calorimetry. The structures and binding affinities of the enzyme-PET complex at the active site were determined with molecular docking calculations and further refined with molecular dynamics simulations to account for flexibility and temperature effects. A gradual increase in the experimentally observed optimum temperature for activity was observed from PETase to HotPETase. HotPETase is able to operate at 65OC but its kinetic stability for PET degradation is significantly lower compared to PETase at 40OC. In most cases, the trajectory analysis of the molecular dynamics simulations identified that at increased temperatures, the probability of PET obtaining the optimum distances within the active site is increased. Finally, HotPETase, at 65OC, was found to have a significantly larger probability of obtaining the necessary distances compared to the predecessors at any temperature.

James Woodward, University of Sussex

Growing complimentary crops and nutritionally rewarding cultivars to sustain insect pollinators and crop pollination on farms

About 70% of crop plants grown globally rely to varying degrees on insects for pollination. These crops are responsible for 35% of worldwide food production and it has been estimated that €153 billion of global food production would have been lost in 2005 if pollinating insects were absent. Wild pollinators have declined in abundance and diversity in North West Europe and North America. Pollination deficits for pollinator-dependent crops have been identified. Optimising pollination for pollinator-dependent crops is of great value to society as these crops produce many fruits, vegetables, seeds, nuts and oils which provide sources of micronutrients, vitamins and minerals for healthy human diets. Therefore identifying strategies to mitigate pollination deficits is of international importance. An approach to boost pollinators and pollination on farmland is to develop complementary cropping systems where sequentially flowering pollinator-dependent crops extend the period of forage availability for pollinators. This strategy could be further enhanced by identifying crop cultivars which are highly nutritious and attractive to pollinators. The benefits of this could be two-fold, improving the reproductive success of pollinators and supporting more efficient crop pollination in current and subsequent years. Identifying nutritious crop cultivars involves quantifying the nutritional quality of the floral resources nectar and pollen. Nectar mainly provides a source of carbohydrates, whereas pollen predominantly supplies proteins, lipids and micronutrients. This

project investigates whether pollination deficits can be mitigated on farmland by selecting nutritious and attractive crop cultivars for complementary cropping systems.

Third-year student talks (2020 cohort)

Fiyin Adenekan, University of Southampton

New Regulators of Mitosis

Mitosis achieves the segregation of duplicated genetic material resulting in two identical daughter cells, which is an essential process in the cell cycle in all higher organisms. Early events from cell rounding and chromosome condensation to attached sister chromatids pulled to opposite poles of the spindle and subsequent cytokinesis rely on specific protein complexes to ensure conditions at each checkpoint are met and prevent erroneous chromatid separation. Most notably, M-phase kinase - CDK1-cyclinB propagates mitosis by activating and inhibiting thousands of proteins. In humans, the loss of this complex results in cells exhibiting mitotic exit defects. Many of these proteins' roles have been characterised, however some remain unknown. A recent phosphoproteomics study revealed uncharacterised substrates of CDK1-cyclinB with a significant fold change following its loss. This project will characterise these proteins' function using a combination of CRISPR-based editing and siRNA with high throughput fluorescence imaging that is followed by in vitro and vivo studies of individual candidates that appear as candidates for novel mitotic regulators.

Charlotte Bilsby, University of Kent

'Plastic eating yeast: the enhancement of Saccharomyces cerevisiae as tool for recombinant protein production'

Plastic is one of the most widely used synthetic material and accounts for one of the most common single use materials worldwide. With only 9% of all plastic materials produced being subjected to recycling, many will enter the natural environment and lead to devastating implications. Its is imperative that alternative means of plastic disposal are explored extensively. Since the 2016 discovery of the novel enzyme, PETase, an expansion into the field of Bio-catalysation of PET materials has been observed. From enhancements of the protein itself to the delivery system of the protein various aspects have been published, however, most applications focus on high yields of the protein being purified from cell lysate, a costly and time-inefficient process, without full degradation of the PET material. We hope to overcome this by using S. cerevisiae, Baker's Yeast, as an

enhanced expression system for the production and application of PETase. Our current approach utilises key biological features of S. cerevisiae such as biofilm formation, alongside our knowledge of codon optimisation of recombinant DNA to increase the effectiveness of PET degradation. So far, we have shown that S. cerevisiae can export active PETase into the extracellular environment, and that different strains of S. cerevisiae can influence the patterns of PET degradation. With this knowledge we hope to improve our protein yield and apply our system to other proteins to help target heterogeneous municipal waste, a huge hurdle in global recycling.

Brandon Coke, University of Southampton

Large scale metanalysis to a develop novel methodolgy for transcriptomics analysis and uncover the key factors for genes' repsonsiveness to perturbation

Previous research performing large scale metaanlysis of transcriptomic data has identified there are a subset of genes with a higher reponsiveness to perturbations increasing their propensity for differential expression. Conventional approaches to omics analysis do not account for this issue and this can confound subsequent analyses when identifying the most biologically relevant differentially expressed genes. Herein, this project has developed a novel approach to account for these intrinsic differences in a gene's responsiveness- GEOrefelct. GEOreflect leverages the large pre-existing transcriptomics datasets to compare differentially expressed gene's statstical significance. GEOreflect then adjust the priotisation genes in the analysis based on this comparison to aid in the interpretability of the results. Application of GEOreflect on studies perturbating key Wnt regulators resulted in increased prioritsation of Wnt associated genes. Additionally, this project is developing a machine learning model to predict a gene's relative responsiveness to stimuli based on genomic and transcriptomic features. The analysis has revealed key features of the untranslated regions and genomic regulatory features which appear to be important for the support vector machine (SVM) model to predict a gene's responsiveness in. Current work now focuses on identifying key signalling pathways and complexes associated with these hyper-responsive genes and incorporating this information into the SVM model via gene ontology node-to-vec embeddings.

David Fisher, University of Southampton

Solving Problems We Can't C: Exploring Seasonal and Regional Micronutrient Insecurities in the UK Food System

The quality of our diets is intrinsically linked with our health and wellbeing. Vitamin C is a well-known micronutrient, synonymous with oranges and touted as a remedy for colds, that plays essential roles in immunity, cognition, and tissue repair. Recent studies highlight the UK's increasing over-reliance on imported fruit and vegetable to provision sufficient vitamin C. Without intervention, a "business as usual" food system stands to seriously jeopardise vitamin C supplies and people's

already deteriorating health. Developing effective interventions will, however, require a more detailed picture of past and present UK vitamin C security. Through collaboration with Kantar World Panel, data describing UK consumers monthly fruit and vegetable purchases between March 2017 and 2022 have been combined with the UK's composition of foods database, population estimates, and import/export quantities, to provide a more granular view of where vitamin C comes from, and where it ends up. A North-South divide in purchases of vitamin C closely resembles recent surveys of household food insecurity, indicating the importance of socio-economic status on accessing vitamin C-rich foods. Some not-so orange fruit and vegetables including peppers, strawberries, and broccoli, dominate popular sources of vitamin C, but their popularity is distinctly seasonal and not always overlapping. Building such complexities into our understanding of vitamin C security is creating a basis for designing targeted interventions that will make a more tangible difference to people's health than a one-size fits all approach.

Laura Freeman, University of Kent

Deciphering the Interactions Between the Gut Microbiome and Innate Immune System in Ageing and Alzheimer's Disease Using the Caenorhabditis elegans Model System

Age is the biggest risk factor of Alzheimer's disease (AD) affecting 7.1% of people over 65 through the oligomerisation and aggregation of the neurotoxic peptide amyloid-β (Aβ), formation of neurofibrillary tangles and neuroinflammation. Biological ageing is accompanied by a reduction in gut microbiota richness and alterations in its composition to favour the colonisation of pathogenic and pro-inflammatory bacteria. Similar changes have been shown in the composition of the AD gut microbiota compared with healthy age-matched controls, suggesting that age-related compositional changes could be causative of AD. This is supported by studies in mouse models showing that the gut microbiota influences AD pathology. An increase in Aβ load following systemic inflammation in rodent models indicates that the innate immune system may play a role in connecting the compositional changes in the ageing microbiome and Aβ deposition. In this study, we use transgenic Caenorhabditis elegans expressing human Aβ to understand the relationship between the gut microbiota, innate immunity and Aβ toxicity. We investigate the hypothesis that the composition of the gut microbiota contributes to AD pathology through the activation of innate immunity and inflammation, causing increased Aβ toxicity. Using a simplified experimental microbiome based on the native C. elegans gut microbiota, we show that bacterial species within our experimental microbiome suppress Aβ toxicity whilst the pathogenic Pseudomonas aeruginosa increases Aβ toxicity, demonstrating that host-microbe interactions alter Aβ toxicity in a C. elegans model.

Isabella Garcia, University of Kent

How does mis-activation of testis-specific genes disrupt mitotic cell division

Mis-activation of testis-specific genes can potentially disrupt downstream miotic cell division. Is this mis-activation resulting in DNA damage, genetic rearrangement, or oncogenesis? More specifically, our research is focused on Zinc finger Y-chromosomal protein (ZFY), a possible transcription factor. ZFY is a gene located on the non-recombining region of the Y-chromosome and has been identified to have two splice variants: a long form that is ubiquitously expressed and a short form that is testis specific. Previous work has suggested a possible cancerous role of ZFY as expression was identified in a Male HNSCC cell line. Could this ZFY expression provides a biological basis for the higher rates of HPV infection and HNSCC in men relative to women? RNAseq work has shown that the overexpression of both the short and long-forms result in high levels of gene differentiation in mammalian cell lines, with ZFYL potentially acting on thousands of genes. ZFYS does not seem to be a transcriptional repressor as it's not a direct antagonist of ZFYL. Though ZFYS does bind to many of the same targets as ZFYL, it seems to function as a weaker transcriptional activator. Work looking at clinical data has shown possible correlations to Head and Neck Cancers, but it is weak and further experimentation is required. Current ongoing work is looking at purifying the N-terminal acidic activating domain of both forms of the protein to do pull-down experiments. Then using our RNAseq data we can establish what are actual targets of ZFY and not just downstream signals.

Hope Haime, University of Sussex

Using transcriptomics to understand the role of DIS3L2 in regulating RNA stability during the cellular stress response

The regulation of RNA stability functions to maintain a fine balance between RNA synthesis and decay which is crucial for key processes involved in cell survival and maintaining cellular homeostasis. Emerging research has suggested a role for RNA stability in regulating the cellular stress response, including the role of exoribonucleases, such as DIS3L2. DIS3L2 is a highly conserved, cytoplasmic 3'-5' exoribonuclease involved in post-transcriptional control of gene expression through targeted RNA decay. Interestingly, our preliminary findings show loss of DIS3L2 in Drosophila melanogaster and human HEK-293T cells confers increased survival and resistance to starvation stress. With the use of CRISPR-Cas9 gene editing, we have generated novel DIS3L2-knockout human cell lines for the controlled manipulation of DIS3L2 expression. Additionally, we have used a range of phenotypical assays to optimise a gradual yet distinct cellular stress response model specific to nutrient deprivation and Endoplasmic Reticulum stress. Using our optimised stress response model, we see a reduction in eIF2α activity and translational activity within our human HEK-293T cells in response to nutrient deprivation. Furthermore, RNA-sequencing is allowing us to investigate the effect of DIS3L2 activity on the interplay between RNA stability and translation of specific transcripts in response to stress. Our preliminary investigations have found distinct RNA

transcripts which show sensitivity to DIS3L2 specifically under nutrient deprivation or ER stress conditions. These findings will allow us to elucidate the key cellular mechanisms that are aiding cell survival during the cellular stress response and the role that DIS3L2 plays within this.

Johanna Haszczyn, University of Southampton

Investigating new approaches of mitigation against organophosphate nerve agents using *C. elegans*

Organophosphorus nerve agents are potent inhibitors of acetylcholinesterase that elicit numerous neurotoxic effects. This acute toxicity arises from the accumulation of acetylcholine at peripheral and central cholinergic synapses. This leads to the cholinergic syndrome, the signs and symptoms of which are attributable to excessive stimulation of muscarinic acetylcholine receptors and nicotinic acetylcholine receptors. Seizures, coma and disruption to respiratory drive occur at high doses, these signs are mediated centrally by both muscarinic and nicotinic receptors and can be lethal. At present, current pharmacotherapy relies on antagonism of muscarinic acetylcholine receptors using atropine, oxime reactivators of organophosphate inactivated acetylcholinesterase and anticonvulsants such as diazepam. Given that the cholinergic syndrome is driven by both muscarinic and nicotinic receptor overstimulation, therapeutic approaches could be improved by countering the excessive stimulation of nicotinic receptors. There is limited use of such pharmacotherapies because of the potent hypotensive responses associated with these drugs. New approaches rely on selective antagonism of the muscle nicotinic acetylcholine receptor with a use-dependent antagonism. It is not yet understood the most efficient sites within the muscle nicotinic acetylcholine receptor for this type of antagonism. Caenorhabditis elegans promises a route to quantify and investigate the intoxication at cholinergic neuromuscular junctions as it provides a full molecular toolkit and a complete gene sequence. This enables the study of new approaches to target the nicotinic acetylcholine receptor using a single point amino acid missense mutation screen that isolates selective sites within the muscle nicotinic receptor that provide the greatest mitigation from organophosphate poisoning. We aim to describe these selective sites to inform new approaches of selective antagonism from organophosphate poisoning.

Steven Houghton, University of Southampton

Analysing the functional significance of a primate-specific glutamate receptor subunit

All aspects of cognitive function require the excitatory neurotransmitter glutamate. One of the main receptors that binds glutamate is the NMDA receptor (NMDAR). NMDARs are Ca2+-permeable and contribute to long-term neuronal changes; playing a role in learning, memory, and neurodegeneration. NMDARs are tetramers within which the GluN2A subunit is incorporated. Our

lab has recently found that a primate-specific GluN2A isoform (GluN2A-S), resulting from alternative splicing, is present abundantly within the human brain (Warming et al., 2019). GluN2A-S is as a shorter protein compared to the canonical GluN2A with a truncated C-terminal domain. As a result, GluN2A-S lacks a PDZ-binding motif and synaptic localisation may be dependent upon the formation of tri-heteromeric NMDA receptors. Expressing human GluN2A results in a mix of protein, preventing study of the isoforms independently. I have generated a plasmid construct that only generates the canonical human GluN2A protein. This was by single nucleotide substitution within the 3' splice site for the rat GRIN2A equivalent site as the rat GRIN2A gene only generates one isoform. I will use it to answer whether GluN2A-S can localise synaptically and whether GluN2A-S contributes similar NMDAR properties. In rat GluN2A the opposing nucleotide change was not sufficient to generate two GluN2A isoforms. With this tool, we can study the impact of GluN2A and GluN2A-S separately in disease-associated GRIN2A mutants; such as the epilepsy-associated L812M missense mutation. Ultimately this work will allow us to gain insight into some of the human-specific intricacies of synapse function and plasticity in health and disease.

Samuel Jones, University of Kent

Enzymology of Vitamin B12 associated enzymes

Vitamin B12 is a complex cofactor that is involved in a variety of biochemical reactions and is essential to human life. Understanding how this molecule is biosynthesised and utalised by enzymes is important for understanding the fundamentals of biosynthesis, B12 dependent enzymatic catalysis and to manipulate the molecule to allow for novel analogue production. Firstly, work has focused on understanding the anaerobic biosynthesis of the lower ligand of vitamin B12. More specifically, work has been carried out characterising BzaC, an O-methyltransferase, required to make a B12 lower ligand intermediate. Furthermore, an alkene containing cobamide intermediate has been generated via co-factor manipulation of the BzaC reaction. In addition, a class of vitamin B12 dependent enzymes, the vitamin B12 dependent radical SAM enzymes, have emerged as significant players in the biosynthesis of many natural products such as antibiotics, bacteriochlorophyll, and anticancer agents, however they remain poorly characterised. To understand more about this new class of enzyme, two Vitamin B12 dependent radical SAMs, CloN6 and BchQ, involved in clorobiocin and bacteriochlorophyll biosynthesis, respectively, have been chosen for characterisation. Although this project is in early stages the aim is to understand more about their reaction condition requirements, structure, and mechanisms.

Liam Jones, University of Southampton

Development of a model system to investigate the effects of surface roughness and media on marine biofilm formation and microbiologically influenced corrosion

The energy sector continues to face corrosion challenges, with significant pipeline failures due to microbiologically influenced corrosion (MIC). This study aims to develop a representative model system in which inoculae relevant to operating pipelines can be cultured to investigate biofilms and MIC on carbon steels. Two identical anaerobic CDC reactors ran simultaneously for 28 days; one inoculated with a multi-species marine consortium and the other uninoculated. Carbon steel (UNS G10180) discs were used with two surface roughness profiles, Ra of 1.33±0.71 µm and 0.44±0.03 µm, as received and polished, respectively. Test media were either artificial seawater supplemented with yeast extract (1 g/L) or ATCC 1249 growth media. Molecular microbiological assessment, plus optical analysis and electrochemical tests were performed. As expected, biofilms have a marked impact on the corrosion mechanism and reactor environment. Sulfide concentrations initially increased in the inoculated reactors (523±118 µmol/L). Additionally, there was a negative shift in corrosion potential, attributed to microbe attachment and biofilm formation/growth. Localised and shallow pits were clearly discernible in the biotic media, whereas only uniform corrosion was evident for the abiotic media. Electrochemical impedance was used to characterize the interfacial properties. This study provides insight into the role of biofilm formation on MIC and the importance of using multiple lines of evidence (MLOE), incorporating a multidisciplinary approach to develop understanding of the mechanistic relationship between the biofilm and metallic degradation. These insights will support a move towards evidence-based biocide dosing and influence recommendations for new industry standards.

Amy Lovegrove, University of Southampton

Microalgal diet species affects investment of shell growth in Pacific oysters

Pacific oysters (Magallana gigas) are heavily cultivated in aquaculture, where they have been fed the same species of microalgae for decades, with Haptophyte Isochrysis galbana being the most common, and Eustigmatophyte Nannochloropsis gaditana also being frequently used. The nutritional value of these algae are well established, however the effect on oyster growth and the underlying molecular mechanisms of this are less researched. Understanding how microalgae affects growth is critical to determining optimal feed species. A feeding trial where 72 M. gigas were fed either mono-specific I. galbana, bi-specific N. gaditana and N. oculata, or an equal mix of all revealed that those fed I. galbana gained significantly more mass than those in the other two diet conditions. Further analysis into Oyster Condition Index showed that whilst they gained the most mass, the I. galbana-fed oysters had significantly lower dry shell weight (DSW) to dry tissue weight (DTW) ratio indicating an investment of energy into biomineralisation. RT-qPCR of several candidate calcification genes suggests that calmodulin (cg_CaM) could be upregulated in I. galbana-fed oysters, and perlucin (cg_Perl) could be down-regulated in oysters fed a mixed species diet. To fully comprehend the pathways that determine biomineralisation, enzymatic analysis of lipid metabolism will be undertaken. Lipids are a critical compound in oyster

development, and these analyses will inform how the microalgae can be enhanced to elicit gene regulation changes in M. gigas.

Letitia McMullan, University of Sussex

How does a mild reduction in oxygen supply constrain hippocampal function?

Cerebral blood flow (CBF), oxygen saturation (sO2), red blood cell velocity (RBCV) and capillary density are lower in the hippocampus than other brain regions with similar neuronal activity; likely making it especially vulnerable to even a mild decrease in blood/ oxygen supply, as seen in early Alzheimer's Disease (AD). However, little is known about how such a mild decrease in blood/oxygen supply affects hippocampal neuronal and neurovascular function, and whether this may lead to subsequent hippocampal dysfunction and cognitive decline in AD. We modelled this in 6 adult male and female C57BL/6J mice. Cortex overlaying the hippocampus was surgically ablated, 2µl AAV1.CaMKII.GCaMP6f.WPRE.SV40 was infused 300µm below the hippocampal surface, and custom-made cannula were implanted over the hippocampus. Net haemodynamic measures, including CBF and sO2 were recorded using a combined laser doppler flowmetry/haemoglobin spectroscopy probe (Oxy-CBF probe). The cerebral metabolic rate of oxygen consumption (CMRO2), a proxy for neuronal activity, was calculated. 2-photon imaging of neuronal calcium signalling was performed. Changes in these parameters in response to lowering the fraction of inspired oxygen (FiO2) via a nose cone from 21% to 19%, 17%, 15%, 13% or 11% for 1 hour were recorded in awake head-fixed mice. Reducing the FiO2 to 17% or lower for 30-45 minutes significantly reduced hippocampal sO2 ($p<0.05^*$), despite increased CBF ($p<0.05^*$). The CMRO2 and frequency of neuronal calcium events were increased during hypoxia ($p<0.05^*$). This suggests excitatory hippocampal neurons may become hyperactive during mild hypoxia.

Kseniia Pidlisna, University of Kent

Investigating Genome Packaging during Recombinant AAV (rAAV) Gene Therapy Viral Vector Production

Gene therapy is an approach to treat diseases by correcting an underlying genetic problem. The usual approach to this involves the delivery of exogenous genetic material into target patient cells to correct the faulty gene causing the disease.Viral vector systems are the most widely used gene therapy delivery vehicles. Of the available viral vector systems currently utilised, Adeno-associated virus (AAV) is often used. AAV is a non-enveloped dependovirus that relies on co-infection with another virus in order to replicate. It is composed of an icosahedral capsid that contains a linear single-stranded DNA genome of about 4.7 kb. The AAV genome encodes several protein products, four non-structural Rep proteins, and three capsid proteins (VP1–3). The production of recombinant AAV is usually achieved via triple transfection of HEK293 host cell lines. The three plasmids co-

transfected usually contain the Rep and Cap genes, a helper plasmid, and the target genome (gene of interest, GOI) flanked by AAV ITRs (inverted terminal repeats). However, production of fully packaged AAV (viral vector containing genome of interest) remains challenging and a major contributor to the cost of gene and AAV therapies (costs can be in the £100,000's to millions per treatment) where often less than 20% of capsids are successfully packaged with the required genome. This project aims to understand limitations upon successful genome packaging of AAV titre and packaging efficiency of GOI into the capsids when produced using HEK293 cells.

Klaudia Piotrowska, University of Southampton

ATM inhibition potentiates cancer immunotherapy by increasing cancer-associated fibroblast-mediated CD8 T-cell infiltration into the tumour microenvironment

The success of immune-checkpoint blockade (ICB) is limited to a fraction of cancer patients, highlighting the need to identify targetable resistance mechanisms to improve its clinical effectiveness. Non-responsiveness to ICB can result from a limited T-cell infiltration mediated by the tumour microenvironment (TME). Cancer-associated fibroblasts (CAFs) are a key component of the TME and due to their heterogeneity CAFs have diverse functions. Myofibroblastic CAFs (myCAFs) have been recently found in the immuno-excluded tumours from patients responding poorly to ICB. Although, myCAFs are associated with poor outcome in most solid tumours, the molecular mechanisms regulating myCAF accumulation remain unclear, limiting potential for therapeutic intervention. Previous findings show that during TGF-β1-induced differentiation, myofibroblasts upregulate a number of genes associated with DNA repair. The aim of this study was to study the role of Ataxia-Telangiectasia Mutated (ATM) in regulating the myCAF phenotype.
To investigate the role of ATM, we treated fibroblasts with TGF-β1 and examined activation of the myofibroblastic markers [SMA, fibronectin, collagens). In vitro, targeting ATM pharmacologically suppressed and reversed myCAF differentiation. In vivo, targeting fibroblast ATM suppressed myCAF-rich tumour growth and promoted intratumoural CD8 T-cell infiltration. Our findings show that ATM inhibition 'normalises' myCAFs by downregulating genes associated with the deposition of desmoplastic stroma rich in collagens and alters the secretome composition, both of which facilitate T-cell movement. This work identifies a novel pathway regulating myCAF differentiation and provides a rationale for using ATM inhibitors to overcome CAF-mediated immunotherapy resistance.

Paige Policelli, University of Southampton

Friendly fire: Identification of cis- and trans-acting elements involved in the regulation of the genome-editing enzyme, APOBEC3A

Background. Aberrant activation of the apolipoprotein-B mRNA-editing catalytic polypeptide-like 3A (APOBEC3A) cytidine deaminase has been implicated as a major source of C>T and C>G

mutations in cancer; in particular in carcinomas of the head and neck, bladder, lung, oesophagus, cervix and breast. We are studying APOBEC3A regulation in a non-cancerous keratinocyte cell line (NIKS) to identify triggers of APOBEC3A expression during cancer development. Building on our recent discovery that APOBEC3A expression is potently induced upon mitogen-induced cell cycle re-entry in NIKS, we aimed to identify the cis- and trans-acting elements involved using Circular Chromosome Conformation Capture (4C) and RNA interference (RNAi). *Methods.* Using chromatin modification data, two bait regions likely representing an APOBEC3A promoter and enhancer were selected. 4C libraries were prepared from proliferating, quiescent and growth factor stimulated NIKS and were sequenced on an Illumina HiSeq 2500. A panel of transcription factor candidates including REL-A, STAT2, c-JUN, FRA-1 and SNAI1 were targeted by short-interfering RNAs to assess the effect on APOBEC3A expression. *Results.* Robust interactions between both baits and multiple regions up- and downstream of the APOBEC3A gene have been detected, some of which (candidate silencers) are enriched in proliferating NIKS (low APOBEC3A expression) and others of which (candidate enhancers) are enriched upon APOBEC3A induction. Furthermore, the siRNA knockdown of multiple transcription factors has identified candidates important for both the abrogation and induction of APOBEC3A. *Future work.* Candidate regulatory regions will be interrogated for transcription factor binding sites, the functional significance of which will be probed using CRISPRCas9-mediated mutagenesis.

Fardina Rahimi, University of Southampton

Genetic basis interspecies oviposition impacting the horticultural pest Drosophila suzukii

Drosophila suzukii (Matsumura) or Spotted Wing Drosophila (SWD) is a fruit fly species native to Southeast Asia, that acts as an invasive pest in other parts of the world. SWD integrated pest management research has focused on finding biological controls against infestation of soft and stone fruit cultures. In this study we examined the deterrence of SWD egg laying by pre-exposure of egg laying substrates to the sister species Drosophila melanogaster, first noted by Shaw et al., (2017) and further characterized by Tsungadi et al., (2022). Experiments using D. melanogaster cultures raised in the presence of antibiotics or following dechorionation at the embryonic stage demonstrated that this interspecies deterrent signal was elicited by the bacterial microbiome of D. melanogaster. Notably, we identified an alternative lab D. melanogaster culture that possessed a bacterial microbiome that attracted rather than repelled subsequent SWD oviposition. Genetic crosses indicated that both the microbiome and the D. melanogaster genotype played a role in determining the valence of the interspecies signal. We will present metagenomics sequencing analyses of repellent and attractive D. melanogaster microbiomes and explain how this information may inform integrated pest management of SWD.

Anne Romero, University of Southampton

Genomic constrains on domestication: Why are so few species domesticated?

Why so few species have been domesticated out of the hundreds that are edible is an important question related to food security. Here we examine the role of plasticity, the ability of the organism to acclimate to different environments, and if it could have aided the domestication of some species over others. We are elucidating the role of gene expression plasticity as mechanisms by which crop progenitors were at a selective advantage over never-domesticated wild relatives. We focus on the tomato crop to explore the gene expression changes between species as well as across environments. Differential gene expression analysis, Gene ontology (GO) analysis and KEGG pathway analysis were performed for leaf and fruit samples. Progenitors showed greater gene expression plasticity than the crop and the never-domesticated wild species. Genes plastic in the progenitor were enriched in several KEGG pathways including those related to hormones signal transduction. Plastic genes in this pathway could have indirect links to various plant processes including plant growth, fruit ripening, stress response and disease resistance. This shows that a subset of the genes that have diverged in expression during the domestication process were those which were initially plastic in the progenitor. Plasticity could have given the progenitor, S. pimpinellifolium, a selective advantage over other wild tomato species in early cultivation. This gives an insight into evolutionary mechanisms during domestication with implications for climate adaptation and food security.

Robert Ulrich, University of Kent

Identification and characterisation of vitamin B12 binding proteins for use in B12 extraction and purification

Vitamin B12 is an important nutrient for maintaining good health and preventing a variety of health problems. While it is found naturally in animal products, such as meat, fish, and dairy, some people need to take supplements or receive injections to ensure they are getting enough of the nutrient. It is also, structurally speaking, the most complex of all the essential 13 vitamins required by humans for good health, and is uniquely made only by certain bacteria. The structural complexity of the molecule negates a chemical synthesis for this important nutrient and hence commercial production relies on highly evolved B12-producing strains of bacteria grown in large-scale fementers that make the commodity in relatively poor yield. However, once produced the nutrient requires purification through chromatographic separation followed by recrystallisation, a prolonged, incremental approach that adds further expense to the overall process. In my project, I have been working on the identification of high-affinity B12-binding proteins with a view to using these on an affinity matrix to rapidly extract and isolate very pure material. My project has led to the identification of a number of novel and hitherto unknown B12-binding proteins, some of which have properties that can lend

themselves to several different biotechnological methodologies, including B12 purification.

Roman Urban, University of Kent

Shedding light on DNA-protein interactions one molecule at a time

The interactions between DNA and proteins are essential for many biological processes, including gene expression, replication, and DNA repair. Single molecule microscopy has revolutionized the study of these interactions by allowing us to observe them at the individual molecule level with unprecedented resolution. By visualizing these interactions, we can uncover new insights into the fundamental mechanisms of life. Furthermore, this enables the quantification of such interactions providing us with protein kinetical parameters including diffusion constants and binding lifetimes. The ability to observe and quantify these interactions in real-time and at the single molecule level has enormous potential for advancing our understanding of complex biological systems. Furthermore, it has the potential to uncover new targets for drug development, as well as new insights into the molecular basis of diseases such as cancer and genetic disorders. In this presentation I will discuss how single molecule fluorescence microscopy can be used to study eukaryotic DNA repair using a combination of techniques such as DNA tightropes and laser tweezers to observe and quantify the interactions between fluorescently labelled proteins and individual DNA molecules. This provides crucial information not only about the interaction of proteins with DNA, but also their partners within a complex or a pathway.

Chloe Uyl, University of Kent

Improving the production of second-generation biofuels by exploiting the natural diversity of the yeast *Scheffersomyces stipitis*

Global warming, high greenhouse gas emissions and depleting reserves of fossil fuels have made it necessary to explore alternative energy sources that are sustainable and renewable in order to meet the world's future energy needs. In particular, greenhouse gas emissions within the transport sector have increased at a faster rate than any other. Biofuels, such as bioethanol are promising alternatives which could reduce dependence on traditional transportation fuels and reduce their associated effects on climate change. Lignocellulose represents the largest renewable source of waste biomass available and can be used to produce second generation bioethanol. Lignocellulose is a complex polymer consisting of cellulose, hemicellulose, and lignin. Hemicellulose, the second most abundant component, is composed of various pentose and hexose fermentable sugars. The well-established fermenting yeast Saccharomyces cerevisiae is usually the microorganism of choice for industrial processes; however, it is unable to naturally ferment pentose sugars (incomplete fermentation). The non-conventional yeast Scheffersomyces stipitis has the highest native capacity for xylose fermentation of any known microorganism. This makes it the most suitable choice for

second generation bioethanol production as it can ferment both pentose and hexose sugars. Different Scheffersomyces stipitis natural isolates vary in their ability to produce bioethanol, but the genetic basis underlying this remains largely unknown. To this end, we are analysing the genomic structure of a collection of S. stipitis natural isolates using both long and short read sequencing techniques. This will allow us to correlate genetic and phenotypic traits to strains with superior bioethanol production.