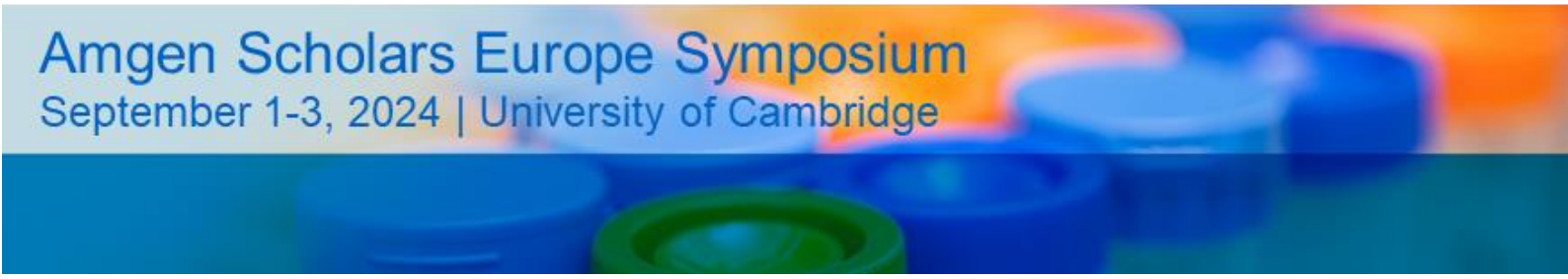

Amgen Scholars Programme 2024

Abstract Booklet

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LMU

Andrew Healy

Home Institution: University College Cork

Host Institution: LMU

Project Area: Cellular Biochemistry

Project Supervisor(s): Professor Dr. Ulrich Hartl

Investigating the role of LONP1 in α-Synuclein aggregation & mitochondrial protein import

Mitochondrial dysfunction and aggregation of α-synuclein are two inextricable features of Parkinson's Disease. Despite this long-understood connection, the mechanistic details linking mitochondrial dysfunction and α-synuclein aggregation remain poorly understood. The mitochondrial AAA+ ATPase LONP1 has recently been identified as a modifier of α-synuclein aggregation. LONP1 has been implicated in various roles in the mitochondria, including clearance of misfolded proteins and import of proteins from the cytoplasm into the mitochondria. Interestingly, recent findings suggest that monomeric α-synuclein can be imported into the mitochondria for degradation. The aim of this project is to assess the role of LONP1 in mitochondrial protein import, as well as to analyze the link between mitochondrial protein import and α-synuclein aggregation.

Mitochondrial protein import was analysed in HEK293 MTS-GFP cells by determining the

colocalization between the matrix-targeted GFP and a fluorescent mitochondrial dye

using microscopy. To analyse whether mitochondrial protein import modulates α-synuclein aggregation, protein import into the mitochondria was inhibited using small molecule inhibitors and RNA interference. The percentage of cells containing aggregates was determined by flow cytometry utilizing HEK293 cells expressing a FRET-based sensor for α-synuclein aggregation, while α-synuclein monomer levels were analysed by western blotting.

LONP1 knockdown decreased mitochondrial protein import, suggesting LONP1 function is vital for this process. Direct inhibition of mitochondrial protein import increased α-synuclein aggregation without changing monomeric α-synuclein levels. Perturbation of LONP1 produced a similar phenotype, indicating that LONP1 does not directly degrade monomeric α-synuclein, but rather suggesting that decreased protein import following the loss of LONP1 function may contribute to α-synuclein aggregation. Further studies will be needed to understand the mechanism at the nexus between LONP1 disruption, mitochondrial protein import inhibition, and α-synuclein aggregation.

Avelina Rybinski

Home Institution: Lund University

Host Institution: LMU

Project Area: Immunology

Project Supervisor(s): Dr. Corinne Benakis

The microbiome in stroke: Rethinking our beliefs about the lung's immune niche

1 in 4 people above the age of 25 will develop a stroke in their lifetime. Despite decades of research, treatment options are limited. Thus, stroke outcome depends heavily on the body's own immune response. Our microbiome and immune cells interact at barrier sites like the gut and lungs, communicating through metabolic signaling and modulating the immune response. A third of stroke patients develop post-stroke pneumonia, significantly increasing the risk of mortality. The cause of this is still unknown as the microbiome-lung-brain axis remains mostly unexplored in stroke due to the lung's small bacterial content.

In this project, we aim to characterize short-term changes in the lung microbiome following stroke using the filament middle cerebral artery occlusion (fMCAo) mouse model.

The lungs were removed from stroke and control mice at 2 days and 3 days following fMCAo. The bacterial DNA was extracted from the homogenized lungs after depletion of host cells from the sample. The final product was then used for quantitative PCR. To visualize bacterial growth, the remaining homogenate was plated on agar plates and assessed after 24h incubation.

Bacterial DNA isolation showed a high interindividual variation in the amount of extracted DNA. Additionally, the fraction of eukaryotic DNA in the lung samples was increased compared to caecum samples, showing the small bacterial niche in the lung compared to the gut. We also found that there is a transient increase in the lung's bacterial population that is strongest on day two post-stroke.

Here, we prove that stroke impacts the lung microbiome and is therefore worth further investigating. Using the isolated bacterial DNA and sequencing, a microbial profile of the stroke lung can be established in the future to identify prominent strains that may impact stroke-related pneumonia and outcome.

Daniel Suñer Rubio

Home Institution: University of Barcelona

Host Institution: LMU

Project Area: Metabolism

Project Supervisor(s): Margherita Springer

Investigating the Role of Peripheral FKBP51 in Glucose Homeostasis

Obesity is a growing global health issue that increases the risk of several diseases like type 2 diabetes. A key feature of both conditions is the development of insulin resistance, which impairs glucose uptake. FKBP51 is a co-chaperone protein primarily known for its role in the

stress response, but recently it was discovered that inhibition of FKBP51 enhances glucose uptake in skeletal muscle, thus inhibition of FKBP51 is a promising approach for regulating glucose homeostasis in the context of obesity and type 2 diabetes.

Given FKBP51's high expression in skeletal muscle, adipose tissue, and brain, this project aims to characterize the specific role of FKBP51 in peripheral tissues by phenotyping a novel transgenic mouse model with FKBP51 deletion in adipose tissue and skeletal muscle (double knockout) using the Cre-Lox system. We compared wildtype and double knockout mice under a chow diet, monitoring weekly body weight and analyzing body composition. Glucose and insulin tolerance tests were performed to evaluate the diabetic phenotype and insulin sensitivity of the mice, respectively. A functional assay was conducted to test if the knockout in muscle influences the coordination of mice. Additionally, we performed an in vitro glucose uptake assay using wildtype and FKBP51 knockout HeLa cells to explore glucose uptake differences between genotypes.

Our results in mice show that FKBP51 double knockout does not result in significant changes in coordination, body weight, and body composition, but it tends to improve glucose clearance. Insulin tolerance test results indicate that this improvement in glucose tolerance is not due to changes in insulin sensitivity, suggesting changes in insulin secretion in double knockout mice.

These findings imply that FKBP51 double knockout has a slight impact on metabolism under chow diet. Future research will explore disease-specific effects of this genotype by inducing obesity and type 2 diabetes in these mice.

Hana Babić

Home Institution: University of Sarajevo

Host Institution: LMU

Project Area: Molecular Oncology

Project Supervisor(s): Ali Ertürk

Optimizing Near-Infrared Visualization in Ultramicroscopy for Enhanced Cancer Metastasis Profiling

Metastasis accounts for over 90% of cancer-related deaths, and existing therapies often fail to effectively target all cancer cells. While current methods enable the detection of fluorescently labelled cancer cells and antibody-based therapies in whole-body mice, we aimed to advance this approach by introducing a fourth channel in the near-infrared (NIR) spectrum. This additional channel would complement the existing ones used for background, cancer, and therapy detection by targeting a new cellular marker. This advancement would provide a more comprehensive view of the tumour-therapy environment.

We focused on optimizing the fourth NIR channel using the Ultramicroscope Blaze, with the mouse intestine selected as a model due to its well-characterized lymphatic vessels expressing the Lyve1 marker. We conjugated Lyve1 antibodies to NIR dyes following established protocols and cleared tissue samples using the SHANEL pipeline.

The experiment consisted of two phases. In the first, Lyve1 primary antibodies were directly labelled with NIR dyes in two of the samples, while in the other two, they were additionally paired with secondary antibodies labelled with 647 nm dyes to enhance signal detection. In

the second phase, the conjugation process was improved by removing harsh chemicals that could damage the antibodies and by adjusting the antibody-to-dye concentration ratio. However, despite these adjustments, both phases yielded negative results, likely due to chemical incompatibilities between the NIR dyes and the antibodies.

These findings suggest that current antibody-dye conjugation techniques are insufficient for NIR imaging applications, indicating the need for further optimization or alternative methods. Successful advancements in this area could significantly improve cancer metastasis profiling and expand the use of ultramicroscopy in other biological fields.

Ilgin Sezer

Home Institution: Koc University

Host Institution: LMU

Project Area: Molecular Medicine

Project Supervisor(s): Prof. Dr. Stylianos Michalakis

Targeting the “stubborn” retina microglia cells using Adeno-Associated Virus (AAV) vectors

Retina microglia cells are immune cells that become activated when there is damage to photoreceptors, causing migration and phagocytosis of degenerating photoreceptors. However, they are often overactivated and destroy healthy photoreceptors as well. This leads to even worse outcomes in inherited retinal disorders (IRD), such as retinitis pigmentosa. Adeno-associated virus (AAVs) vectors are ideal for targeting specific cells in vivo and the gold-standard for gene therapy approaches, also in the retina context. Hence, the current study focuses on how AAVs can be modified to target retina microglia cells, known to be refractory to transfection and transduction methods. To achieve this aim, a primary mouse retina microglia cell culture was developed. Cells were transduced with AAV vectors that have modified capsids and carry eGFP as transgene. After five days, we observed microglia cells transduced with the AAV GL.R variant have the highest percentage of GFP expression among other variants. Furthermore, to restrict the expression of the vector to our target cells, the microglia/macrophage-specific CD68 promoter was evaluated. CD68-GFP plasmid was transfected to microglia and non-microglia cell lines. We found that in non-microglia cell lines (HEK293 and MEF) the expression of CD68-GFP was significantly reduced compared to the expression of GFP under a universal promoter; however, there was no significant difference in GFP expression in the microglia cell line (HMC3). Based on these promising results, the CD68 promoter was truncated to accommodate the limited packaging capacity of AAV vectors. In the future, AAV vectors with a microglia-targeting capsid and a cell-restricted promoter will be produced to deliver genes to retina microglia cells to overcome overactivation and treat retinal diseases.

Lea Kojičić

Home Institution: Faculty of Mathematics, University of Belgrade

Host Institution: LMU

Project Area: Neuroscience

Project Supervisor(s): Andreas Herz

DUMB: Decoding Uncertainty Meets Bayes

Neural decoding, the process of reconstructing sensory stimuli or motor intentions from neural activity, is pivotal in understanding how the brain interprets and represents information. This task is inherently challenging due to the noise and variability present in neural responses, which differ even when the same stimulus is presented multiple times. Traditional approaches like Population Vector (PV) decoding estimate the direction of the stimulus based on the collective activity of neuronal populations but lack a mechanism to gauge the confidence in these estimates.

To address this limitation, we incorporated a measure of uncertainty into the PV decoding process. Specifically, we used the magnitude of the PV as an indicator of the reliability of the decoded estimate—a larger PV magnitude suggests stronger, more consistent neuronal alignment in a particular direction, correlating with higher confidence in the result.

In this project, we initially implemented standard PV and Maximum Likelihood Estimation (MLE) decoding methods and observed how the introduction of noise, simulated through neural dropout, deteriorates decoding precision. To improve accuracy, we adopted a Bayesian approach inspired by Brown et al (1998), which combines prior knowledge with observed evidence. By computing posterior distributions over possible estimates using neural activity at consecutive time points, and integrating information from both PV decoding and a refined extrapolation method, we were able to merge these distributions using MLE to achieve a more robust and accurate final estimate.

Mariia Riabova

Home Institution: King's College London

Host Institution: LMU

Project Area: Epigenetics/Molecular Oncology

Project Supervisor(s): Prof Dr Axel Imhof

Decoding the Methylome: Accelerating Drug Discovery via Mass Spectrometry

KDM1B, a lysine-specific histone demethylase, plays a crucial role in regulating gene expression by removing methyl groups from histone proteins. Dysregulation of KDM1B has been implicated in various cancers, where it can lead to abnormal gene silencing or activation, thereby promoting tumorigenesis. This study aims to dissect the molecular functions of KDM1B in cancer by employing a multifaceted approach that includes proteomic, metabolomic, methylation and histone profiling.

Using CRISPR/Cas9 technology, we generated KDM1B knockout (KO) HEK 293 cells to investigate the downstream effects of KDM1B loss. Through deep proteome analysis (DIA-PASEF) and LC-MS/MS, we identified significant alterations in protein expression and

metabolic pathways associated with the absence of KDM1B. Our data highlight the enzyme's role in modulating key processes such as methylation, phosphorylation, and cellular metabolism.

Methylation profiling revealed specific changes in the methylome of KDM1B KO cells, providing insights into how the loss of KDM1B influences epigenetic landscapes and contributes to cancer progression. These findings suggest that KDM1B is a vital regulator of epigenetic modifications and underscore its potential as a therapeutic target in cancer treatment.

Future research will focus on further elucidating the impact of KDM1B on the cellular composition by integrating additional assays such as the use of demethylase inhibitors and advanced methylation analysis techniques like Oxford Nanopore sequencing.

Müge Öztürk

Home Institution: Istanbul Medipol University

Host Institution: LMU

Project Area: Molecular Oncology

Project Supervisor(s): Prof. Dr. Olivier Gires

From Light to Insight: Unraveling Invasive HNSCC with Dendra2 Photoconversion

Head and neck squamous cell carcinomas (HNSCC) are malignancies of the upper aerodigestive tract, associated with significant morbidity and a high global mortality rate. Patients often present with nodal metastases at diagnosis and frequently experience relapses after the primary tumor treatment. Cellular differentiation programs such as the epithelial-to-mesenchymal transition (EMT), driven by epithelial growth factor receptor (EGFR), are shown to enable tumor cells to acquire the functions essential for migration, local invasion and metastasis. While treatments targeting EGFR, such as cetuximab, are available and commonly used in the treatment of HNSCC, their therapeutic effectiveness is limited due to the absence of biomarkers for response prediction, challenges in patient stratification and development of drug resistance. Therefore, a deeper understanding of EGFR-mediated EMT is in demand.

In this project, we aimed to develop cellular models that replicate cetuximab resistance in HNSCC to investigate the associated molecular and transcriptomic changes. We utilized a 3D spheroid-based model that mimics carcinoma in situ and local invasion of HNSCC cells and integrated it with the photoconvertible Dendra2 tracer protein. The accuracy of our model was verified using FACS analysis and fluorescence microscopy. We also developed an alternative cell line with cetuximab resistance to verify our findings from the Dendra2 model.

In the next step, we plan to photoconvert and isolate invasive cells treated with EGFR ligand EGF, as well as those treated with both EGF and cetuximab, that remain invasive despite cetuximab treatment. We will then perform RNA sequencing on the isolated invasive cells from both models and use bioinformatics to identify differentially expressed genes (DEGs). Our ultimate goal is to identify genes associated with cetuximab resistance that could serve as biomarker candidates for response prediction or even novel therapeutic targets, potentially optimizing treatment strategies for HNSCC patients.

Pablo Fernández García

Home Institution: Universidad Autónoma de Madrid

Host Institution: LMU

Project Area: Neuroscience

Project Supervisor(s): Christian Peters, Rüdiger Klein

Fasting Modulates the Role of CeA Projections to PSTN in Feeding Behaviour

The Amygdala is well known for its role in fear and emotional responses. However, this highly conserved brain region is involved in several other functions such as social behaviour, reinforcement learning and feeding behaviour. More specifically, the Central Amygdala (CeA), composed solely of inhibitory GABAergic neurons, is known to play an important role in hedonic feeding, driven by "pleasure" rather than by homeostatic needs.

Recent work published by the Klein's Lab shows that Htr2a neurons, a CeA neuron subpopulation, project to the Parabrachial Nucleus (PBN) and that their activation enhances feeding. Moreover, preliminar results also show that the CeA also sends projections to the Parasubthalamic Nucleus (PSTN), a region whose activation has proven to diminish food intake. Furthermore, fasting activates Htr2a neurons and hyperpolarises PSTN neurons. Thus, given that CeA neurons are GABAergic, we hypothesise that CeA neurons projecting to PSTN are Htr2a neurons and that their activation enhances feeding by inhibiting PSTN neurons.

After carrying out different chemogenetic manipulations of this projection in mice, retrograde viral tracings and immunostainings, we found that activating this projection decreases food intake rather than enhancing it and that it is not the Htr2a subpopulation that we hypothesised that projects to PSTN. Most interestingly, the effect of inhibiting this projection depends on whether the mice have been fasted or not. These results open many questions and elucidate the complexity of neural networks and their role in behaviour.

Rebecca Florea

Home Institution: Università di Pisa | Sant'Anna School of Advanced Studies

Host Institution: LMU

Project Area: Neuroscience

Project Supervisor(s): Sowmya Narayan | PI - Prof. Mathias Schmidt

Sex-dependent responses to developmental stress and psilocybin

Background: Developmental stress exposure increases the risk of stress-related disorders in adulthood, such as depression. Given the limited long-term efficacy of many antidepressants, researchers are increasingly exploring the potential of psychedelics, such as psilocybin, which has been shown to reopen the critical period for social reward learning in mouse models. However, the sex-dependent effects of developmental stress and psilocybin intervention remain understudied.

Methods: We studied 94 mice, first stratified by sex, and then randomly allocated to eight groups based on prenatal early-life stress exposure and psilocybin intervention. Behavioural tests were conducted three days after psilocybin or vehicle administration. Mouse

behaviours were phenotyped using the machine learning-based programs DeepLabCut and DeepOF. Principal Component Analysis and Integrated Z-score approaches were used to assess internalizing and social behaviours, reflecting depressive-like phenotypes and socialization tendencies. Statistical tests included two-way ANOVA for each sex, with the categorical variables “stress treatment” and “psilocybin intervention,” or three-way ANOVA on all animals, adding “sex” to the previous categorical variables.

Results: Statistical analysis revealed a significant increase in internalizing behaviours among stress-exposed mice compared to controls (three-way ANOVA: $p=0.002$). Psilocybin treatment significantly reduced internalizing behaviours (three-way ANOVA: $p=0.0005$) and increased social behaviours (three-way ANOVA: $p<0.05$) compared to vehicle administration. When stratified by sex, psilocybin significantly decreased internalizing behaviours in males (two-way ANOVA: $p<0.0001$) but had no significant effect in females. Conversely, it significantly increased social behaviours in females (two-way ANOVA: $p=0.005$) but not in males.

Conclusion: Our results suggest that developmental stress induces a depressive-like phenotype in adult mice. Psilocybin effectively reduces depressive-like behaviours in males and enhances sociability in females, highlighting the importance of considering sex differences in therapeutic interventions.

Siebe van Manen

Home Institution: Utrecht University

Host Institution: LMU

Project Area: Sensory Neurobiology

Project Supervisor(s): Prof. Dr. David Keays

The Cellular Architecture of a Magnetosensory Circuit in Pigeons

Magnetoreception is the sense that enables animals to detect the Earth’s geomagnetic field. While a large body of behavioural studies supports the existence of magnetoreception across a broad range of species, the primary sensors, underlying mechanisms, and neuronal basis of the sense remain unknown.

Previous experiments in the Keays lab have identified increased neuronal activation in the medial vestibular nuclei, dorsomedial thalamus, and mesopallium of pigeons upon exposure to a rotating magnetic stimulus. However, the cellular architecture of these activated areas remains largely unexplored. Therefore, we aimed to optimise a fluorescent in situ hybridisation protocol for pigeon brain sections to subsequently map the distribution of major neuron classes within these areas.

The gene expression of major neuronal classes in the pigeon brain was successfully spatially validated using the optimised multiplexed fluorescent in situ hybridisation chain reaction. Cellular distributions corresponded with those found in closely related avian species, allowing the visualisation and validation of distinct brain structures.

These findings provide a first insight into the cellular architecture of the pigeon magnetosensory circuit. In the future, this optimised method can be applied to spatially validate novel marker genes of neuronal subtypes derived from single-nucleus RNA sequencing data, allowing more detailed investigations of the pigeon brain. Combining this

method with activation experiments could enable the characterisation of magnetosensory neuronal populations, advancing our understanding of the molecular mechanisms underlying magnetoreception in pigeons.

Sofia Hu

Home Institution: Trinity College Dublin

Host Institution: LMU

Project Area: Biochemistry

Project Supervisor(s): Karoline Kadletz, Ceren Kimna, Ali Ertürk

LipiGo: A novel strategy for the functionalisation of lipid nanoparticles

Lipid nanoparticles (LNPs) are currently used in the clinic as efficient nucleic acid carrier systems, e.g. Pfizer's COVID-19 mRNA vaccine. In this project, we propose a new strategy to functionalise LNPs in order to achieve enhanced targeting. This strategy was utilised to produce LNPs conjugated to AS1411, a DNA aptamer. Multiple conjugation conditions were tested including varying reaction temperatures, reaction buffers, and targeting ligand to LNP ratios. Conclusive evidence from Dynamic Light Scattering and Electron Microscopy indicate that LipiGo is a functional method to conjugate targeting ligands to the surface of LNPs. Biological validation studies were carried out to assess cellular uptake of LNPs in vitro. The model system encapsulated mRNA tagged with a fluorescent dye allowing detection by confocal fluorescence microscopy. The functionality of LipiGo was tested on model lung cancer cell line A549, as these cells overexpress nucleolin, the protein that is targeted by the AS1411 aptamer. As nucleolin is overexpressed in a range of cancers, this LNP-AS1411 conjugate could be utilised in cancer treatment in future. Future work will involve in vivo biodistribution studies, comparison to standard methods for LNP functionalisation, and the application of LipiGo novel linker technology to a variety of targeting ligands to achieve a broad range of targeting properties.

Institut Pasteur

Anja Matic

Home Institution: École Polytechnique

Host Institution: Institut Pasteur

Project Area: Bioimage Analysis

Project Supervisor(s): Thibault Lagache, Raphael Reme

Temporal Star-Dist: Improving Segmentations Methods for Fluorescent Images of Hydra Vulgaris

A promising method to break the neural code, that is to relate the activity of neurons to function, is fluorescent labeling of neurons and time lapse imaging of animals such as Hydra Vulgaris, the fresh-water polyp. To analyze neuronal activity, neurons first need to be identified and due to the large number of neurons, an automatic segmentation method is required. In addition, fluorescent images of Hydra present challenges, such as overlapping

objects, variation in brightness and blur caused by movement, that current methods are not equipped to deal with. To solve these challenges a novel method, Temporal Star-Dist, is proposed. It is based on the Star-Dist method, which is an established deep-learning method for segmentation of cells and other objects in biological imaging. The key difference with the new Temporal Star-Dist method is the exploitation of temporal information. Taking a pair of images related in time, and optionally the computed optical flow, as input, the deep learning model predicts a pair of segmentations. In this work the potential of the method is demonstrated on a dataset containing 7 pairs of images from 4 different image sequences of Hydra, annotated by hand, where the protein TdTomato is labeled. Training our models using five different splits of the dataset does not show general improvement compared to Star-Dist. However, in particular cases where images contain overlapping objects and motion blur, we do observe improvements. This shows that Temporal Star-Dist is a promising method to tackle the challenges Hydra images pose but that work remains to reach the full potential and to fully utilize the temporal information as well as the optical flow. In the future, Temporal Star-Dist could be integrated with tracking and used with other types of Hydra images.

Carla Zou Yin Rodríguez Rubio

Home Institution: La Salle - Ramon Llull University

Host Institution: Institut Pasteur

Project Area: Biological Image Analysis

Project Supervisor(s): Jean-Christophe Olivo-Marin

Deep Wavelet Detector: enhancing segmentation of neurons in fluorescent images of Hydra Vulgaris

The detection and segmentation of neurons in fluorescent microscopy images present significant challenges, particularly when dealing with the freely behaving Hydra vulgaris organism. These difficulties arise from the small size of the neurons in the images, the substantial variation in their brightness, and the frequent blurring caused by the organism's movement. While there are established pre-trained models for segmentation tasks in biological imaging, their performance in Hydra imaging has proven insufficient, leading to suboptimal results. To address this issue, the current study introduces a novel approach aimed at enhancing the detection and segmentation results. The proposed method integrates the captured images with their undecimated wavelet transforms and utilizes this enriched dataset to train an improved StarDist model for more accurate predictions. The dataset is created by extracting regions of interest of 200x200 pixels from seven pairs of frames taken from videos capturing the Hydra in motion. These frames are manually annotated to establish the ground truth necessary for training and validating the model. The model's performance is then evaluated against both the fine-tuned StarDist and Wavelet detector models, each trained solely on the dataset images without additional modifications. The proposed method demonstrates improved detection and segmentation results compared to the other models. This advancement represents a significant step forward in developing more robust systems for modeling Hydra vulgaris behavior and could have broader applications in addressing other challenges in biological imaging.

Charlotte Duda

Home Institution: University of Fribourg

Host Institution: Institut Pasteur

Project Area: Cell Biology

Project Supervisor(s): Dr. Serge Bonnefoy and Dr. Aline Alves / Dr. Philippe Bastin

Investigating I17 as a landmark in Trypanosoma brucei for the positioning of axonemal microtubule doublets

Cilia and flagella are cell organelles that have essential roles in motility, sensing and development. The axoneme is the core structure of cilia. It is well conserved among eukaryotes and composed of 9 microtubule-doublets (dMT) that surround a central pair of single microtubules. In Trypanosoma brucei, the causative agent of African Trypanosomiasis, the axoneme is physically linked to an extra-axonemal structure, the Paraflagellar Rod (PFR). Using transmission electron microscopy (TEM) images, dMT can be numbered according to their orientation relative to the central pair and the PFR: dMT1 is opposite to the PFR, which is slightly parallel to the central pair microtubules. However, due to the diffraction limit of light microscopy, the precise definition of individual doublets is not possible. This can be overcome by expansion microscopy and identification of protein markers. In this project, we investigated I17, a repetitive protein identified by TEM to be located between the PFR and the axoneme and presumably on one of the fibers that links the PFR to the axoneme either on doublet 4 or on doublet 7. With tagged I17 cell lines we first established the appropriate primary and secondary fluorescent antibodies. Subsequently, we performed expansion microscopy where the position of I17 respective to the axoneme and the PFR was observed. To gain higher resolution, promising experiments using the iterative ultrastructure expansion microscopy (iU-ExM) approach have been initiated to identify the exact localization of I17.

Elisa Baas

Home Institution: Université de Strasbourg

Host Institution: Institut Pasteur

Project Area: Immunology

Project Supervisor(s): Eric Legrand (Supervisor) / Chetan Chetnis (PI)

Development of a blood stage mRNA vaccine for Plasmodium falciparum malaria

Malaria is an infectious disease caused by the transmission of the parasite plasmodium,through the bite of an Anopheles mosquito. Despite several curative treatments, two of the five species capable of infecting humans, plasmodium falciparum (Pf) and plasmodium vivax (Pv), still account for over five hundred thousand deaths each year. The majority of those deaths are caused by Pf, placing the finding of an effective vaccine against it as a great necessity. Current vaccines, RTS,S and R21, target CSP protein, which enables the sporozoites to enter the liver. However, due to the parasite's complex life cycle, a multistage vaccine would be more effective. In order to develop a blood stage mRNA vaccine, and because previous results suggest that their immunogenic effect could be

additive or even synergistic, two proteins, PfRH5 and PfTRAMP, have been selected. Both are parts of the PCRCR complex, used by the parasite to enter red blood cells. Several constructs of mRNA for these proteins have been created. Using HEK cell transfection, western blot and immunofluorescence assays, we have been able to select two constructs that are expressed and presented on the cell surface. Based on these results, a co-transfection of both PfRH5 and PfTRAMP mRNA constructs could be implemented. This blood-stage target in Pf could then be incorporated into other stages of the life cycle or even applied to other parasites of other species, such as Pv.

Daniella Bakoli Sgourou

Home Institution: University of Patras

Host Institution: Institut Pasteur

Project Area: Stem Cell Biology

Project Supervisor(s): Guillaume Burnet, Luis Altamirano Pacheco/ Pablo Navarro

Uncovering mitotic bookmarking properties of selected factors in embryonic stem cells

Introduction: Mitosis is accompanied by chromatin changes and general transcription silencing. In fast cycling cells, such as mouse Embryonic Stem Cells (mESCs), the correct transcriptional network must be promptly re-established following mitosis to ensure cell identity preservation. One hypothesis is that a class of Transcription Factors (TFs) remains bound to their interphase targets during mitosis and favour their fast transcriptional reactivation, effectively playing the role of a ‘bookmark’. To identify novel bookmarking TFs, we analysed genomic regions that retain histone acetylation (generally associated with transcription) better during mitosis and correlate with faster gene reactivation. Enrichment of binding motifs for YY1, E2F4 and FOXH1 in those regions led us to test their bookmarking activity. In addition, we also aimed to evaluate mitotic binding of the epigenetic reader BRD4, known to associate with acetylated histones and TFs.

Materials &Methods: For all factors, we generated mESCs lines ectopically expressing GFP fusion proteins. We performed live-imaging of mitotic cells to identify TF localization during mitosis. We then performed Chromatin Immunoprecipitation sequencing (Chip-seq) for all factors in asynchronous and mitotic mESCs, in two replicates, to identify retention of site-specific interactions in mitosis.

Results: In live-imaging YY1 is consistently enriched at the centromeres, while BRD4 shows modest chromosomal enrichment. ChIP-seq analysis showed around 10% of their interphase targets retains binding in mitosis for both factors, confirming bookmarking activity. The bookmarked regions display higher acetylation levels than non-bookmarked, both in asynchronous and mitotic cells. Genes near bookmarked regions are reactivated faster upon mitotic exit. Analysis of FOXH1 and E2F4 is ongoing.

Discussion: YY1 and BRD4 are bookmarking factors in mESCs, although the small number of replicates we used limits the accuracy of the analysis. Correlation of bookmarked peaks with higher acetylation levels and faster reactivated genes is suggestive of a functional significance of the bookmarking activity.

Hristina Milojkovic

Home Institution: Faculty of Biology, University of Belgrade

Host Institution: Institut Pasteur

Project Area: Cell Biology of Host-pathogen interactions

Project Supervisor(s): Klementina Borovnik / Agathe Subtil

Reshaping intracellular traffic and cellular architecture by bacterial proteins

Chlamydia trachomatis is an obligate intracellular pathogen that survives in a special membrane-bound compartment called inclusion inside a host cell. It secretes various effector proteins to hijack the host cell's compounds, including an Inc protein family located in the inclusion membrane. In chlamydial infection, the intracellular vesicular trafficking, regulated mainly by the Rab GTPases, is hugely affected. Rabs bound to GTP are active and bound to membranes, while Rabs bound to GDP can be sequestered in the cytosol. Rab11 plays an important role in vesicle trafficking, including transferrin recycling, and it is recruited to the inclusion by an unknown chlamydial partner during the early stages of infection. In one of the previous experiments in our laboratory, it was observed that upon Chlamydia trachomatis serovars D IncS protein overexpression, Rab11 cellular distribution may become more cytosolic. Following this observation, our project aimed to observe Rab11 cellular distribution in the IncS presence. Cells were transfected with constructs for fluorescently labelled IncS and Rab11, fixed, stained with DAPI and analysed with immunofluorescence microscopy. Due to Rab11's role in transferrin recycling, another part of the project was to observe transferrin's cellular distribution in IncS's presence. The workflow was similar - cells were transfected with IncS construct, went through live uptake of fluorescently marked transferrin, fixed, stained with DAPI and analysed with immunofluorescence microscopy. Results indicate that the cellular distribution of Rab11 and transferrin is shifted to a more cytosolic one upon IncS overexpression. However, further experiments should be conducted to quantify these interactions. The interaction of IncS and Rab11 will be assessed through a subcellular fractionation experiment, where cell lysates of cells co-transfected with IncS and Rab11 will be separated in membrane-bound and cytosolic fraction by ultracentrifugation, enabling the Western Blot comparison of IncS and Rab11 abundance in the fractions.

Marcel Nos Llorenç

Home Institution: University of Barcelona

Host Institution: Institut Pasteur

Project Area: Developmental Biology

Project Supervisor(s): Dr. David Green

Understanding the Genetics of Neuroblast Delamination

Epithelial-mesenchymal transition plays a key role in development and tumorigenesis. It entails the loss of apicobasal polarity and cell adhesion leading to delamination of the cell. During *Drosophila melanogaster* embryogenesis neural stem cells (neuroblasts, NB) undergo an EMT-like process (ingression) where the apical domain is reduced and the NB delaminates basally. Genetically encoded positional cues define a stereotyped pattern of proneural genes, specifically the achaete-scute complex (AS-C), in groups of cells forming

proneural clusters. This pattern is refined via Notch-mediated lateral inhibition ensuring a single cell per cluster maintains proneural expression and adopts a NB fate. It is unclear whether the AS-C plays a direct role in ingression. We have used a combination of fixed antibody stains and live imaging of AS-C deficient embryos to resolve this question. We confirmed previously published results using fixed antibody stains of AS-C deficient embryos, showing that immature NB can be observed under the ventral epithelium, which suggests that they delaminate in the absence of the AS-C. To test whether AS-C deficiency has any effect on ingression dynamics we crossed AS-C mutant females with males carrying a *DEcad*-GFP reporter, allowing visualisation of adherens junctions to track cell size and shape. Then, we imaged the neural ectoderm during NB ingression and performed single embryo PCR to determine their genotype. While this project is ongoing, our preliminary results do not show a statistically significant difference in ingression speed. Our current data suggests that NB ingression proceeds mostly normally in AS-C deficient embryos, despite other NB abnormalities, which may indicate that proneural genes do not play a major role in regulating the initiation of ingression, though additional work must be conducted to confirm this.

Marie-Batisse Heite

Home Institution: TU Delft

Host Institution: Institut Pasteur

Project Area: Cell Biology

Project Supervisor(s): dr. Nassim Mahtal

Early detection of apoptosis using High-Content Analysis (HCA)

High-Content Analysis (HCA) uses both automated image analysis and automated microscopy to simultaneously measure multiple phenotypic parameters of samples, limiting bias. This approach helps profiling cells and to reveal significant and subtle patterns. Here, the overall objective of the project is to investigate how increasing the number of parameters analyzed through HCA impacts the accuracy and the feasibility of early detection of phenotypes, by focusing on apoptosis as a well-known model. Presented work will cover the first steps of the project. HeLa cells were treated with apoptotic-inducing compounds, solvents and/or fluorescent probes to optimize protocols and assess cytotoxicity. The latter was evaluated through a common resazurin assay where its intensity is measured with a plate reader, and through image-based nuclei count. Data showed that both approaches are equivalent. Additionally, dilutions of probes and compounds demonstrated that within the tested concentration range, there was no evidence of probe-induced cytotoxicity, and optimal concentrations of drugs were identified. Overall, these data allows us to go further and explore multi-parametric image analysis.

Mireia Puig Seguí

Home Institution: Polytechnic University of Valencia

Host Institution: Institut Pasteur

Project Area: Cell biology

Project Supervisor(s): Sevan Belian

The role of LIM kinases in regulating the formation of functional tunneling nanotubes

Tunneling nanotubes (TNTs) are non-adherent, open-ended, thin actin-based protrusions connecting cells over distances up to hundreds of microns and enabling cytoplasmic continuity and the transfer of various cellular components (such as ions, RNAs, proteins, and entire organelles). They have raised significant interest due to their roles in the spread of misfolded protein aggregates involved in neurodegenerative diseases and in conferring resistance to chemo- and radiotherapeutic treatments in cancer cells. This research project aims to explore wether LIM kinases (LIMKs), key regulators that inactivate through phosphorylation the filamentous actin (Factin)-cleaving protein Cofilin, thereby leading to F-actin stabilization, influence TNT formation and vesicle transfer. It has been reported that stresses known to increase LIMK activity can increase TNT formation and TNT-mediated transfer. Thereby, we aimed to understand if LIMKs could regulate TNT formation in mouse neuronal cells (CAD cells) in the presence and absence of stress inductions. Using western blot and spinning disk confocal microscopy, we transfected CAD cells with functional and non-functional LIMK2 (isoform absent in CADs). We then demonstrated that the overexpression of the functional LIMK2 led to increased cofilin phosphorylation and formation of functional TNTs, whilst the non-functional LIMK2 had the oposite effect due to a downregulation of the total pool of endogenous LIMK1. Additionally, a positive correlation between LIMK1 phosphorylation and the formation of functional TNTs was found upon different stress conditions: serum deprivation, H2O2 and Nitric Oxyde (NO). In parallel, the inhibition of LIMKs appeared to reduce the effects of H2O2 and NO on TNTs, suggesting LIMKs might represent a common regulatory pathway for the formation of TNTs in stress conditions. Such results might help us better understand intercelular communication in pathological contexts and lead the way for the development of new therapeutical approaches.

Romina Fanchiotti

Home Institution: Humanitas University - Medtec School

Host Institution: Institut Pasteur

Project Area: Biology - Biological Image Analysis

Project Supervisor(s): Giacomo Nardi

Morphodynamic modelling of Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are crucial for generating all blood cell types and self-renewing their population, making them valuable for treating various malignant and non-malignant conditions through transplantation. However, challenges like immunological

rejection and donor scarcity limit the effectiveness of HSC therapies. Therefore, research into generating HSCs in vitro is essential to fully realize their clinical potential.

The biological background of this study focuses on understanding HSCs emergence in zebrafish embryos, a model for vertebrate hematopoiesis, using confocal microscopy. Specifically, it investigates the Endothelial-to-Hematopoietic Transition (EHT) within the hemogenic endothelium of the dorsal aorta. During EHT, the endothelial membrane bends and invaginates, leading to the formation and release of HSCs into the bloodstream. This process is driven by transcription factors, like RUNX1, and mechanical forces from the actomyosin belt.

This study introduces a novel method for reconstructing three-dimensional (3D) models of emerging HSCs from confocal microscopy images. This approach uses a topology-based image segmentation algorithm, grounded in persistent homology theory, followed by a meshing algorithm.

The method was validated against experimental videos, ensuring the 3D reconstructions were consistent with the 2D video frames and the temporal evolution of the endothelium. It proved to be precise and topologically consistent in detecting objects within microscopy images, and it demonstrated flexibility in parameter adjustments.

This research represents a significant advancement in visualizing HSC emergence, particularly luminal membrane invagination, laying the groundwork for biophysical and mechanical analyses. These insights could enhance the understanding of HSCs emergence process, eventually improving HSC-based therapies effectiveness in clinical practice.

Veneta Petkova

Home Institution: Sofia University “St. Kliment Ohridski”

Host Institution: Institut Pasteur

Project Area: Genetics

Project Supervisor(s): Pedro Dorado-Morales

Creating a tool for genetic modification of Gram positive bacteria

The emergence of multidrug resistant Gram-positive (G+) pathogens has highlighted the need for a better understanding of the evolution and biology of these medically important bacteria. Functional genomic studies in G+ bacteria have traditionally been limited by the impossibility of genetically manipulating most clinical isolates. To adequately study these organisms, it is necessary to develop methods to overcome the physical and enzymatic barriers that limit the transfer of foreign DNA. Conjugation has been used as a simple and efficient means of transferring DNA in vitro between Gram-negative (G-) species, however, it has not been thoroughly explored in G+ bacteria. The aim of this project is to exploit the conjugation machinery of a broad-spectrum G+ conjugative plasmid as a means to mobilise a shuttle plasmid, from E. coli to a G+ recipient. For that we separated the genes coding for the conjugative apparatus and the origin of transfer of the plasmid pIP501. To efficiently select for the bacteria that received the plasmid, we constructed donor strains auxotrophic for diaminopimelic acid, an essential molecule for the formation of the bacterial cell wall, containing the mobilisable and helper plasmids. To set up the optimal conditions for plasmid transfer, we initially used a wildtype E. coli MG1655 as recipient. Preliminary results showed no observable mobilisation of the plasmid containing the origin of transfer. Future work will

explore the use of other conjugative systems and the development of a G+ donor.

Karolinska Institutet

Giulia Orlando

Home Institution: University of Pisa - Sant'Anna School of Advanced Studies

Host Institution: Karolinska Institutet

Project Area: Metabolism

Project Supervisor(s): Paul Petrus

The liver clock: how chronic jet lag disrupts circadian rhythms and influences metabolism in mice

The liver clock is crucial for nutrient metabolism and energy homeostasis as the main liver functions are rhythmically regulated by clock-controlled genes. Chronic jet lag (CJL) influences the peripheral clock leading to metabolic disorders, but much about the underlying mechanisms remains unknown. This study aims to explore the effects of CJL on metabolism and liver circadian transcriptome in mice. Ten-week-old C57BL6J male mice were divided into four groups according to their diet and lighting conditions: chow diet and normal lighting conditions (CD-NL); high fat diet and normal lighting conditions (HFD-NL); normal chow diet and jet lag (CD-JL); high fat diet and jet lag (HFD-JL). Body weight was measured weekly [n=20/group] and after 8 weeks of intervention, oral glucose tolerance test was performed [n=10/group]. After 9 weeks liver tissues were collected and qPCR for clock-controlled and metabolic genes was performed [n=5/time-point, n=30/group]. Results showed that CJL caused significant weight gain in CD-JL group and impaired glucose tolerance in HDF-JL and resulted in loss of circadian oscillations of clock-controlled genes in CD-JL (Nr1d1), in HFD-JL (Per1, Per2), or in both (Bmal1, Clock, Cry1, Dbp). Metabolic genes under CJL showed alterations of circadian oscillation (Fabp4, Dgat1) and gene expression levels in CD-JL (Fabp4, Lipe) and in HFD-JL (Fabp4, Dgat1), thus affecting lipid metabolism. These results should be taken into consideration for future studies to better understand mechanisms behind metabolic disorders led by CJL.

İdil Büyükgölcigezli

Home Institution: Hacettepe University

Host Institution: Karolinska Institutet

Project Area: Immunology

Project Supervisor(s): Supervisor: Claire Marsal / PI: Evren Alici

Optimization of Nucleofection of iPSC for Testing a Transposon-Based Doxycycline-Inducible System

NK cells differentiated from induced pluripotent stem cells (iPSC) can provide a homogenous source of NK cells for cancer immunotherapy. Unfortunately, the effector functions and surface markers of iPSC-derived NK differ from those of primary NK. Transcriptional

modulation of the differentiation of iPSC to NK cells is hypothesized to improve the maturation of the iPSC-derived NK cells. The aforementioned transcriptional modulation involves the nucleofection of the iPSC with a pair of plasmids that together carry out inducible expression of NK-related transcription factors when exposed to the antibiotic Doxycycline. One of the plasmids in this system carries VENUS, a green fluorescent protein, and the transposase enzyme for the stable integration of the other plasmid's content into the iPSC genome. In this project, we optimized the conditions for nucleofection of the iPSC with this first plasmid, assessing the efficiency of nucleofection via measuring the percentage of VENUS positive cells with a flow cytometer at days 2 and 4 post nucleofection. Of the two reaction volumes we tested for the nucleofection (20 vs 100 µL), we concluded that the smaller reaction volume leads to a higher nucleofection efficiency. We tested different amounts of plasmid DNA (2, 4, 6, 8, 10 µg) to nucleofect 1.10^6 cells. The live cell count of the iPSC after the nucleofection indicates that the nucleofection of plasmid DNA is a clear stressor on the iPSC, exerting a toxic effect. While increasing the amount of plasmid DNA provides a higher efficiency of nucleofection, it comes at the cost of reduced cell counts and viability for the iPSC. We concluded that the optimal amount of plasmid DNA to nucleofect 1.10^6 iPSC is 5 µg to get the best nucleofection efficiency and limit cell death. This optimization is significant for the experiments to progress with the testing of the Doxycycline-inducible system.

Larysa Vynohradnyk

Home Institution: Taras Shevchenko National University of Kyiv

Host Institution: Karolinska Institutet

Project Area: Fertility Preservation

Project Supervisor(s): Amirhesam Eskafinoghani, Kenny Rodriguez-Wallberg

Early effects of cancer treatment on prepubertal testicular tissue: an animal model study

Cyclophosphamide (CPA) is an alkylating agent commonly used in chemotherapy for cancer treatment. The application of this drug for prepubertal males frequently disrupts normal spermatogenesis and can lead to impaired fertility or infertility in the future. While sperm cryopreservation is a viable option for adults, it is not applicable to prepubertal boys since they do not yet produce mature spermatozoa. This project aims to find the mechanism behind gonadotoxic effects of cancer treatment on prepubertal testis. 11 B6/CBA F1 mice male pups (12-day-old) were randomly assigned into control (n=6) and CPA (n=5) groups. They were injected with one dose 100 mg/kg CPA or PBS (control groups) and sacrificed at different timepoints. After tissue dissection, the testis tissues were examined using Histology assessments (H&E, IHC, IF) and RNA sequencing techniques. RNA sequencing revealed differences in gene expression in the prepubertal testis between control groups and those treated with cyclophosphamide (CPA) after 48 hours, particularly in pathways related to apoptosis. Histological analysis and immunohistochemistry showed that cancer treatment with CPA led to a reduction in relative cell numbers, particularly of spermatogonial stem cells (SSCs) in the prepubertal testis 48 hours after treatment. At the same time, immunohistochemistry confirmed the RNA sequencing results, showing an increase in the number of apoptotic cells in the CPA-treated group compared to the control group. Results show that one of the main adverse effects on testis from CPA is reduction of SSCs by activating apoptotic pathways.

Matúš Grieš

Home Institution: Masaryk University

Host Institution: Karolinska Institutet

Project Area: Nanoparticle Chemistry

Project Supervisor(s): Andrea C. del Valle/Georgios A. Sotiriou

Hydrophobic Interactions of Polyphenol Molecules for Self-Assembled Nanoparticles

Many bioactive compounds have significant therapeutic potential but face challenges due to their hydrophobic nature, resulting in poor solubility and bioavailability. Novel drug delivery systems are needed to address these limitations. In this study, we developed a method for preparing quercetin nanoparticles using the zwitterionic properties of folic acid, which forms π-π interactions with quercetin, to improve the solubility of these hydrophobic molecules. We employed dynamic light scattering and zeta potential measurements, along with Fourier-transform infrared spectroscopy, to characterize the prepared nanoparticles. To quantify quercetin and folic acid within the nanoparticles, we optimized a high-performance liquid chromatography separation method combined with UV-Vis spectroscopy. Our results showed that adding folic acid increased nanoparticle size but did not significantly affect stability. Polyphenol quantification indicated that these compounds were retained in the nanoparticles with molar ratios similar to the initial formulations. As a future direction, the precise determination of the water solubility of the quercetin-folic acid nanoparticles and subsequent in vitro testing of their antioxidant activity is suggested. This work lays the foundation for a novel hydrophobic drug delivery system utilizing quercetin-folic acid nanoparticles, potentially applicable to various therapeutic treatments.

Mila Škorić

Home Institution: University of Belgrade

Host Institution: Karolinska Institutet

Project Area: Immunology

Project Supervisor(s): Lola Boutin, Margareta Wilhelm

Immunosuppressive soluble factors produced by medulloblastoma and their effects on activation of γδ T-cells

Medulloblastoma (MB) is the most prevalent malignant brain tumor in the pediatric population. Conventional treatments frequently lead to severe and irreversible neurological and endocrinological deficits. Consequently, there is a critical need to develop therapeutic strategies that are both less toxic and more effective such as γδ T-cells immunotherapy, a promising means of targeting tumours with a low mutational burden, such as MB. However, MB creates an immunosuppressive environment producing factors e.g. TGF-β1 and PGE2 that can have a negative impact on anti-tumor activity of γδ T-cells. The primary objective of this research is to determine the levels of TGF-β1 and PGE2 produced by 7 MB cell lines (DAOY, UW228-3, ONS-76, D425, D458, CHLA-01, CHLA-01R), using ELISA assay and decipher the effects of MB-conditioned media on expanded γδ T-cells. The cell lines were cultured in both monolayer and spheroid structure. After preconditioning them with MB media the antitumor activity and activation marker expression (NKG2D, DNAM-1, Granzyme

B, CD107a) of γδ T-cells were assesed by flow cytometry. The findings were validated using Celecoxib (COX/PGE2 inhibitor) and a TGF-β1 neutralizer. High levels of TGF-β1 and PGE2 were detected in DAOY and ONS-76 cell lines, particularly in spheroids. γδ T-cells conditioned in MB media with higher levels of immunosupressive factors showed lower expression of NKG2D and DNAM-1, whereas Granzyme B was overexpressed in all the samples compared to the media. In all the lines, except for the CHLA-01R, an unexpected increase in CD107a levels was detected. Despite the high levels of TGF-β1 and PGE2 detected in MB conditioned media, it was not sufficient to suppress activation of γδ T-cells. Moreover, the observed increase in Granzyme B and degranulation capacity indicates that MB cells may produce additional agents that could stimulate cytotoxicity of γδ T-cells rather than suppress their activity.

Milena Stepić

Home Institution: University of Belgrade

Host Institution: Karolinska Institutet

Project Area: Molecular Oncology

Project Supervisor(s): Xia Hao/Kenny Rodriguez-Wallberg

Cellular Senescence Role in Ovarian Damage Induced by Cyclophosphamide

Cyclophosphamide (CPA) is a chemotherapeutic agent widely used for cancer treatment. As such, it may lead to ovarian toxicity, premature ovarian insufficiency, and later-life infertility. This therapeutic can induce DNA damage, which leads to cell cycle arrest. Recent studies have highlighted crucial roles of cellular senescence, a type of permanent cell cycle arrest, in developing various diseases. In the present study, we investigated whether cellular senescence is involved in the post-treatment effects of CPA using a mouse-derived ovarian granulosa cell line (KK-1). The cultured cell line was exposed to 4-hydroperoxycyclophosphamide (4-HC), a liver metabolite of CPA prodrug. Cells were cultured for one month. Senescence markers were analyzed using immunofluorescence (p53, p21, β-galactosidase) and Western blot (p21, β galactosidase, cleaved-PARP). Morphological alterations associated with senescence were monitored through mitochondrial alterations (MitoTracker), nuclear diameter measurements and cell count assessment. Treatment with 4-HC upregulated p21, p53, β-galactosidase at 10 μM and downregulated p21, β-galactosidase, cleaved-PARP at 5 μM, slowed cell growth in both concentrations and increased nuclei diameter at 10 μM. MitoTracker staining revealed mitochondrial fragmentation, elongation, and aggregation, indicating disrupted mitochondrial dynamics. Our findings confirmed the presence of cellular senescence in granulosa cells after CPA treatment at 10 μM and may suggest complex regulatory interactions involving p53 and p21. There is an unanticipated signal decrease observed at 5 μM concentration, possibly due to a more complex pathway that bypasses p53. Future research should validate these results in vivo, explore the role of p53 in senescence beyond p21, and employ electron microscopy for detailed mitochondrial analysis.

Raea Michie

Home Institution: Trinity College Dublin

Host Institution: Karolinska Institutet

Project Area: Immunology

Project Supervisor(s): Benedict Chambers

Defining the phenotype of JAML-positive tumor-infiltrating natural killer cells

The junctional adhesion molecule-like (JAML) protein is a transmembrane surface receptor expressed on various immune cells and facilitates cell migration through binding of its ligand, coxsackie and adenovirus receptor (CXADR). JAML acts as a costimulatory protein on CD8+ and γδ T cells. JAML is thus a potential therapeutic target for cancer treatments. However, we have found that JAML is expressed on a subset of intratumoral NK cells. Therefore, this project sought to characterize JAML+ tumor-infiltrating NK cells.

NK cells are critical for immunosurveillance and elimination of virally infected and cancerous cells. NK cell activity is mediated by activating (Ly49D) and inhibitory (Ly49I) receptors. Using spectral flow cytometry data, we found that JAML+ NK populations isolated from Yumm1.7 tumors tended to have low levels of Ly49D and Ly49I, suggesting that these Ly49 molecules could potentially control expression of JAML. Upon further phenotyping, JAML+ NK cells also expressed CD49a, suggesting tissue residency. Finally, JAML+ NK cells also expressed 4-1BB, another NK cell costimulatory molecule.

We also tested the cytotoxic capacity of JAML+ NK cells against MTAP1A tumor lines with high (J+) and low (J-) CXADR expression. When we tested sorted JAML+ NK cells, we found no differences in their ability to kill our different sublines of MTAP1A.

These findings suggest JAML+ NK subpopulations have a distinct profile characterized by low levels of specific Ly49 receptors and association with some markers of tissue residency and activation. Further research is needed to elucidate the impact of JAML on NK cell activity and create future therapies.

Ramona-Delia Mera

Home Institution: Constructor University Bremen

Host Institution: Karolinska Institutet

Project Area: Molecular Genetics

Project Supervisor(s): Dr. Sherwin Chan

The Story of OLA1/YchF Translation Inhibition: Novel Fuctional Insights of Ribosomal Factors

OLA1/YchF is a universally conserved NTPase that was previously shown to play a role in mediating adaptation to stress conditions by influencing translation and protein degradation pathways. Novel structural data have shown that OLA1 is able to bind to mature, uncharged tRNAs. The OLA1-tRNA complex interacts with, but does not completely fit into, the A-site of the large ribosomal subunit in the NDP-bound form, protruding into the decoding center. This observation has led to the hypothesis that the complex may function as a nutrient-sensing mechanism that alters translation under stress conditions. To explore this hypothesis, YchF,

a homologue of OLA1, was overexpressed in BL21 E. coli cells under the control of the T7 promoter, and the effects were compared to those of endogenous YchF levels. The growth curve analyses proved that YchF overexpression in vivo alters bacterial growth patterns, by stunting their growth in the exponential phase, but promoting survival in the stationary phase. Additionally, a pulse experiment using click chemistry indicated that YchF overexpression inhibits protein synthesis. To assess the nature of the YchF effect on translation, an in-vitro translation assay has been performed. The assay was performed in a media that was rich in uncharged Trp-tRNAs, and the translation rate of either GFP or Trp-free GFP constructs was investigated. Despite the interaction of YchF with the decoding center, its effect is not codon-specific; however, when codon specificity is also present, the inhibition is more pronounced. In conclusion, experimental and structural data provide novel insights into the function of YchF as a nutrient-sensing factor that facilitates adaptation to starvation-induced cellular stress.

Roshni Banerjee

Home Institution: Erasmus University Rotterdam

Host Institution: Karolinska Institutet

Project Area: Immunology

Project Supervisor(s): Lisa Westerberg

Cytoskeletal dynamics in immunodeficiency diseases: CRISPR/Cas9-mediated knockout of actin regulators in T cells

The actin cytoskeleton is essential for the integrity of all cells. The dynamic equilibrium between polymerisation and depolymerisation of filamentous actin provides a structural framework for various cellular processes, including cell migration, adhesion, pathogen internalisation, intercellular communication, intracellular signalling, and cell division. In immune cells, impaired actin regulation is implicated in primary immunodeficiency disorders, particularly Wiskott-Aldrich Syndrome (WAS). This study focuses on the genes encoding three critical actin regulators: Wiskott-Aldrich Syndrome protein (WASp), actin-related protein complex subunit 1B (ARPC1B), and non-muscle myosin II A (MYH9). We screened primary human T cells from four healthy donors to evaluate T cell yield and assessed their CD4+/CD8+ ratio and activation state using flow cytometry. Following donor selection, we generated mutant cell cultures of primary human CD4+ T cells using CRISPR/Cas9-mediated gene editing, achieving nucleofection frequencies between 83% and 93%. Inference of CRISPR Edits (ICE) analysis of purified mutant genomic DNA, conducted via PCR and Sanger sequencing, revealed knockout efficiencies of 33% for ARPC1B, 88% for MYH9, and 83% for WAS. Subsequently, we used functional antibodies to activate and expand the modified T cells, followed by analysing protein expression through Western blotting. The targeted proteins exhibited higher expression levels than predicted by their respective knockout scores, suggesting a potential selective advantage of wild-type cells. These results verify the efficient and activation-neutral gene editing of primary human T cells, enabling further functional studies under diverse conditions, including microgravity.

Tudor-Fabian Troncea-Sandu

Home Institution: Carol Davila University of Pharmacy and Medicine

Host Institution: Karolinska Institutet

Project Area: Neuroscience

Project Supervisor(s): Supervisor- Patricia Muñoz PhD, PI- Ivan Nalvarte associate professor

Estrogen deficiency-induced signs of insulin resistance in ovariectomized APPNL-F and APPNL-G-F knock-in mice

Alzheimer's Disease (AD) is a neurodegenerative disorder that affects more than 55 million people worldwide, representing 60-70% of all cases of dementia. Women, especially after menopause, bear a larger burden of the disease, running a two-fold risk of developing AD. Acting as a neuroprotective agent, estrogen is a master regulator of bioenergetic systems in the brain. Furthermore, there is a growing consensus indicating that insulin influences cerebral bioenergetics too. Having an important role in proteostasis, insulin can influence clearance of amyloid β peptide and phosphorylation of tau, which are telltale signs of AD.

To investigate the relationship between estrogen deficiency and insulin resistance, the ovariectomized APPNL-G-F and APPNL-F knock-in mouse models of AD were used. We also aimed to extend our knowledge regarding brain carbohydrate metabolism using SH-SY5Y cell cultures, especially fructose metabolism, as HCFS foods could be a contributing answer to why AD is a disease mostly limited to humans.

6 months old APPNL-G-F mice expressed a decrease in estrogen receptor beta (Esr2) expression, suggesting a paradoxal increase in estrogen levels, APPNL-F presenting the opposite effect. Our hypothesis is that the brain presents a failsafe system of estrogen production for it to become ultimately overloaded in APPNL-F 18 months old mice. Moreover, changes in Glut4 and Igf1 mRNA expression overlapped with insulin resistance specific biomarkers. Preliminary similar results in SH-SY5Y cells cultured in high-fructose concentration media were obtained.

Moving forward, for more accurate results there is a need to create an aging timeline, comparing APPNL-F and APPNL-G-F mice separetly, and to assess the levels of more biomarkers and amyloid β peptide levels in the SH-SY5Y-APPNL cell cultures. The lack of neurological symptoms associated with neurodegeneration in the mice calls for the use of a more biosimilar model, such as iPSC-derived hippocampal spheroids.

Xenia Tzavara

Home Institution: University College London

Host Institution: Karolinska Institutet

Project Area: Microbiology

Project Supervisor(s): Aleksandra Wielento, Amir Ata Saei

Oral-gut microbiome axis: Interactions between oral pathogens P. gingivalis and F. nucleatum and colon cancer cells

Changes in the microbiome are associated with many disease states, including periodontitis and various types of cancer. The oral cavity and the gut are microbial hotspots with significant species overlap, and oral pathogens that can survive the migration through the stomach may colonize the gut, linking oral dysbiosis with changes to the gut microbiome. Porphyromonas gingivalis and Fusobacterium nucleatum are oral pathogens known to interact with each other to promote dysbiosis and inflammation in the gums of periodontitis patients, and increased abundance of these bacteria in the gut microbiome is also associated with colorectal cancer. The aim of this study was to investigate the interactions between the oral pathogens P. gingivalis and F. nucleatum and colon cancer cells, to better understand the connection between oral pathogens and colorectal cancer. Both bacteria were found to invade colon cancer cells, F. nucelatum at a higher rate than P. gingivalis. Infection of colon cancer cells with the bacteria reduced cell viability, and culturing the cells in media containing bacterial supernatant reduced cell proliferation at higher supernatant concentrations. qPCR analysis showed no change in the cells' expression of TGFβ following stimulation with the bacteria, though additional genes involved in inflammation, angiogenesis, or metastasis must be investigated for a more complete understanding of the bacteria's influence on the tumor microenvironment. These results show that the oral pathogens P. gingivalis and F. nucleatum can influence colon cancer cell growth and viability in vitro, providing a basis for future studies investigating their mechanistic role in CRC progression.

ETH Zurich

Ambar Kolari

Home Institution: University College London

Host Institution: ETH Zurich

Project Area: Molecular Biology and Neuroscience

Project Supervisor(s): Prof. Dr. Gerhard Schratt and Simon Galkin

Target-directed microRNA degradation of miR-503-5p

The long non-coding RNA MALAT1 has been previously shown to regulate the levels of the microRNA miR-503-5p, but the exact mechanism underlying this relationship has yet to be elucidated. This project hence explores the possibility that target-directed microRNA degradation (TDMD) is at the root of this interaction. During TDMD, the canonical miRNA-target transcript dynamic is reversed such that the miRNA gets degraded instead. In order to

study this, the levels of MALAT1 in HEK293T cells were manipulated through various systems, including CRISPRa (dCas9-VP64) and an overexpression construct for upregulation, and CRISPRi (dCas9-KRAB), Cas13-NES/NLS and dCas13-based target site blockers for downregulation. The effect on miR-503-5p levels was then measured through a dual luciferase assay. qPCR was also conducted to verify that MALAT1 levels were changing appropriately according to each up- or downregulation system. Overexpression of the MALAT1 TDMD trigger site causes miR-503-5p levels to significantly decrease, which is consistent with miR-503 being sensitive to TDMD triggered by MALAT1. The series of knockdown experiments have raised further questions in terms of the possibility of MALAT1 having varying effects on miR-503-5p levels in a cell cycle dependent manner. This finding would be of relevance in multiple contexts, both in health and disease, as MALAT1 is responsible for the regulation of a wide array of genes at the transcriptional and post-transcriptional level, and has been linked to synapse formation and a variety of pathologies. miR-503 is known to play a role in embryonic development, as well as could potentially be involved in regulating neuronal activity.

David Walley

Home Institution: University College Dublin

Host Institution: ETH Zurich

Project Area: Surgery, Orthopaedics, Biomechanics

Project Supervisor(s): Professor Jess G. Snedeker

Revolutionising Post-Caesarean Uterus Repair: Novel Surgical Technology for Enhanced Healing.

Caesarean deliveries, though common, can lead to serious complications due to impaired healing, largely driven by current suturing techniques that cause tissue damage and limit oxygenation. The University of Zurich and ZuriMED Technologies AG have introduced a new technique, the FiberLocker System, to improve healing by reducing suture-related damage and providing a stable regenerative scaffold. This study compares the biomechanical integrity of uterine tissue using traditional suturing versus the FiberLocker method. In this study, sections of sow uterine tissue were tested under various conditions: naïve; two sutures alone; two sutures with a polyethylene terephthalate (PET) patch; two sutures with a carboxymethyl cellulose (CMC) patch; two sutures with a dry PET/CMC patch; and different combinations of wetted PET/CMC patches. The patches were applied to the perimetrial surface over the sutures and fiberlocked to the tissue. The tissue samples were then subjected to uniaxial tension testing to measure key biomechanical properties: maximum force, ultimate tensile strength (UTS), gapping point, elongation at UTS, and Young's modulus. Results showed differences between traditional suturing and the fiberlocking method, as well as between different patch combinations. Maximum force ranged from 25.2N (two sutures) to 31.1N, with the highest performance in the PET/CMC patch with fluid on tissue. The gapping point varied significantly, with the PET/CMC patch showing the greatest resistance. While no statistically significant difference was found in maximum force, the FiberLocker System demonstrated potentially improved uterine biomechanical strength, particularly in gapping resistance, which could reduce risks like uterine rupture and excessive bleeding, thereby mitigating associated complications. Further biological and biomechanical testing is necessary to fully evaluate the FibreLocker system's potential as a comprehensive repair tool.

Emanuele Rossi

Home Institution: Scuola Superiore Sant'Anna

Host Institution: ETH Zurich

Project Area: Engineering and Data Analysis

Project Supervisor(s): Elisa Donati (supervisor)/ Giacomo Indiveri (PI)

Exploring touch: pattern recognition applied to human and artificial grasp

Touch plays a fundamental role in exploring the reality around us. Our hands allow us to grasp, manipulate objects and infer their material features applying the right amount of pressure. However, the state-of-the-art artificial hands lack tactile feedback and the mechanisms through which humans process tactile information are still unknown.

We want to analyze and classify different types of human grasp (cylindrical, lateral and precision) when both the artificial and the natural hand are handling different kinds of objects. The main focus is to compare the natural hand and the artificial one in order to understand which areas are the most involved in the grasp process.

In order to achieve this, we extracted data-frames through the STAG sensory glove both from the natural hand and the artificial one (MIA hand). Then we preprocessed the data-frames to select the most informative ones for each recording. To analyze the data we finally performed principal component analysis (PCA) for visualization and support vector machine (SVM) for grasp and object recognition.

The results obtained show great performance in the grasp recognition (around 98% of accuracy) but, on the other hand, SVM leads to overfitting when applied to object identification . Moreover, the preprocessed images highlight different patterns of activation for the three types of grasp and these patterns can be more easily identified in the artificial hand’s data-frames rather than in the natural hand’s ones.

Ioana Harabagiu

Home Institution: Constructor University Bremen

Host Institution: ETH Zurich

Project Area: Cell Biology

Project Supervisor(s): Yves Barral

Nipping it in the Bud: Bik1-Kar9 interaction and its role in nuclear positioning in Saccharomyces cerevisiae

In Saccharomyces cerevisiae, accurate nuclear positioning during mitosis is vital for proper chromosome segregation. The microtubule plus-end tracking proteins (+TIPs) Bik1, Kar9, and Bim1 play key roles in this process, orchestrating spindle alignment along the mother-bud axis. This study investigates the molecular interaction between Bik1 and Kar9, focusing on the specific amino acid residues in Bik1 and Kar9 that are crucial for their interaction. Combining genetic mutagenesis with widefield microscopy, we aimed to determine which residues in the linker region of Bik1 are essential for its interaction with Kar9. Moreover, we employed mutant strains and analyzed the effect the mutation of MNIKP conserved region of Kar9 had on its recruitment in the presence and absence of the Bik1 gene. Interestingly,

mutation of the conserved MNIKP patch of Kar9 and deletion of Bik1 led to decreased Kar9 recruitment at microtubule plus-ends, indicated by the reduced fluorescence intensity in mutant strains. These findings stress the importance of the MNIKP patch in the Bik1-Kar9 interaction during mitosis. Future studies will focus on further dissecting the molecular details of this interaction and its broader implications for mitotic accuracy in yeast by investigating which residues of Bik1 crosslink with Kar9.

Mamen Cortés Navarro

Home Institution: CEU San Pablo University

Host Institution: ETH Zurich

Project Area: Biomedical Engineering and Neurotechnology

Project Supervisor(s): Giacomo Indiveri - PI; Elisa Donati - Direct Supervisor

Think Two's A Crowd? Unveiling the power of dual-tasking on muscle-brain connectivity

After a stroke, motor impairments often come with cognitive challenges, yet rehabilitation therapies typically address motor and cognitive training separately, despite their close connection. Studies suggest that a combined approach could speed up recovery and improve brain-muscle connections. But should cognitive and motor tasks be related, or can they be entirely separate tasks performed simultaneously? Our project aims to explore how related and unrelated dual motor-cognitive tasks impact brain-muscle communication in healthy individuals.

The experimental protocol involves four tasks: a motor task, a cognitive task, a related dual task, and an unrelated dual task. The motor task is continuous hand gripping, while the cognitive task uses the Stroop test, a well-known psychological test. The related dual task combines these with slight modifications, and the unrelated dual task replaces the Stroop test with counting backward.

To measure brain-muscle connection, we record electroencephalogram (EEG) and electromyogram (EMG) signals simultaneously during all four tasks to calculate corticomuscular coherence, which measures brain-muscle signal synchronization.

So far, we have made significant progress by designing and refining the protocol and developing the interface needed for the experiments. We are still collecting data, so no conclusions yet, but we expect stronger coherence during the related dual task compared to the others. Stay tuned for our latest results!

If the results align with our expectations, we will demonstrate that related dual tasks can facilitate motor movement, paving the way for improved rehabilitation therapies.

Matilde Bisi

Home Institution: Scuola Superiore Sant'Anna Pisa

Host Institution: ETH Zurich

Project Area: Neuromorphic engineering

Project Supervisor(s): Chiara De Luca / Giacomo Indiveri

Characterization of a hardware-compatible hebbian learning rule

Spiking Neural Network (SNN) is a class of Artificial Neural Network that emulates the behaviour of biological neurons. In SNNs, both input and output signals are represented as spike trains and the model incorporates biological quantities and parameters. The advantage of this approach is the potential to develop hardware implementations of the network using analog electronic components, resulting in applications characterised by low power consumption, low latency, and the ability to perform offline computing. Our aim is understanding the role of certain parameters on the synapse weight update (learning process) of a single neuron in order to find working points or modalities. Our model represents a bicompartmental neuron that can be stimulated from the basal and apical parts, and the main biological quantities modelled are apical and basal currents, postsynaptic firing rate (FR) and calcium current (ICa). We performed simulations varying the stimulation frequency and the ICa thresholds that define whether the synapse strength can be potentiated and/or depotentiated. We observed the evolution of output FR, synaptic weight and calcium current. The main results concern the mean weight change (dw) in function of ICa, the weight changes at different basal, apical and postsynaptic FRs, and the shape of ICa/dw graphs at certain stimulation rates. In all these cases the parameters influence the learning behaviour. We can conclude that by appropriately selecting the neuron's parameters, it is possible to influence the weight update mechanism of the synapse, thereby forcing the neuron to learn in a specific manner according to the desired requirements.

Nataliia Burmistrova

Home Institution: Kyiv National University

Host Institution: ETH Zurich

Project Area: Biology of the cell nucleus

Project Supervisor(s): Maximilian Seurig - Amgen Project Supervisor, Karsten Weis - PI

Influence of Sro9 truncations on heat-shock rescue

Quiescence, a temporary non-proliferative state, is essential for the survival of yeast cells and can be induced by acute glucose starvation. Respiration plays a crucial role in maintaining quiescence, as evidenced by the reduced regrowth capacity of cells treated with Antimycin A following glucose starvation. Previous studies have demonstrated that pre-heat shock extends the regrowth period in respiration-deficient and glucose-starved cells, while the deletion of the SRO9 gene diminishes this benefit. Additionally, the Sro9 protein has been found to significantly enhance reporter expression.

This study investigates the impact of various Sro9 truncations on the heat-shock rescue. Using a spotting assay, yeast cultures were grown to the log-phase, then divided into four

groups, with two groups subjected to heat shock. This was followed by Antimycin A treatment and glucose starvation.

Our results indicate that the heat-shock benefit is reduced in the Sro9-(1-251) and Sro9-(1-151) truncation mutants compared to control strains. Additionally, the growth rates of all Sro9 truncation mutants are lower than those of the wild type, with growth patterns similar to those observed in sro9Δ strains. Furthermore, the expression level, measured by the mean intensity of mNG, varies across all strains.

Nuria Balbás

Home Institution: UFV (Universidad Francisco de Vitoria)

Host Institution: ETH Zurich

Project Area: Biomedical Engineering

Project Supervisor(s): PI: Prof. Dr. Ralph Müller, Advisors: Dr. Friederike A Schulte, Jack J Kendall

Under Pressure: Simulating 3D Osteoporotic Bone's Remodeling Response

Osteoporosis, commonly referred to as the “silent disease”, increases the risk of bone fracture, contributing to an estimated 8.9 million fractures annually. In vivo studies have demonstrated that osteoporotic bone models subjected to mechanical loading exhibit an increased rate of remodeling. The primary objective of this study is to elucidate the molecular mechanism governing bone remodeling under mechanical loading by employing a micro-Multiphysics Agent-Based (micro-MPA) model, and to validate the findings against in vivo data. To this end, the model mainly focuses on the Wnt signaling pathway, regulated by sclerostin production, as the principal response to mechanical stimuli. The model underwent calibration and parameter optimization through a systematic pipeline encompassing data processing, cell viability assessment, and iterative debugging. The results offer a detailed visualization of regions with elevated sclerostin expression and areas of heightened mechanical signaling within a 3D representation of murine vertebrae, showing how these zones visually correspond to areas of bone resorption and formation, respectively. Furthermore, the study provides a comparative analysis of bone density responses observed in silico and in vivo, along with insights into cellular dynamics. In conclusion, the model has demonstrated a successful integration of the potential mechanical loading pathway, sclerostin can be observed as a biomarker for bone resorption, the optimal parameters have been established and the model has proven to be a robust tool for simulating vertebral remodeling in response to mechanical loading.

Petar Damjanović

Home Institution: School of Electrical Engineering, University of Belgrade

Host Institution: ETH Zurich

Project Area: Biomedical Engineering: Movement Biomechanics

Project Supervisor(s): Dr. Deepak Kumar Ravi, PI: Prof. Dr. William R. Taylor

Help me heart, I'm falling: Assessing coordination between cardiovascular and locomotion parameters during gait perturbations

Every year a significant number of older adults experience falls, which leads to health issues and increased mortality, placing also a heavy burden on the healthcare systems worldwide. Resilience, the ability to recover from perturbations, is crucial for maintaining balance and preventing falls. Our body's systems are highly interconnected and work together when the body encounters challenges. However, understanding of these mechanisms, which could eventually result in some practical tools for enhancing resilience, is very limited. We investigate how perturbations during walking influence the coordination between the cardiovascular and locomotor system, aiming to uncover their combined role in maintaining movement stability. The metrics of coordination we use is the coupling coefficient: the product of coherence and cross-spectral density (CSD) of signals of inter-step intervals (ISI) and RR intervals. RR intervals are derived from 1-lead ECG signal while ISI is derived from IMU data, both recorded by Movesense Flash Sensor. Six healthy participants, all under 30, walked on a treadmill for 8 minutes at 4 km/h in four scenarios: walking with perturbations (2.25 m/s^2) versus walking without perturbations, and low-intensity (1 m/s^2) versus high-intensity (2 m/s^2) perturbations, induced by instantaneous treadmill accelerations, occurring every 15 seconds. We observe higher coupling coefficient at the frequency of perturbation both when the perturbations are present compared to normal walking and when the perturbations are more severe compared to the less severe ones. Possible reasons for such occurrence may include increase in physiological coordination when body encounters a challenge, but also the change in trend of the analyzed signals after every perturbation. As conclusion, we hypothesize that by conducting the same experiment on older adults, lower coupling coefficient would be observed due to their lower resilience to perturbations, which could rule out the possibility that observed higher coefficient is not a result of physiological coordination.

Rumeysa Gulcihan Cakmak

Home Institution: Bahcesehir University

Host Institution: ETH Zurich

Project Area: Clinical Biomechanics and Imaging

Project Supervisor(s): Prof. Dr. William R. Taylor

Automated and radiation-free scoliosis screening: bend forward!

Scoliosis, a spinal deformity, necessitates early detection and intervention to prevent progression. Traditional screening involves the Adam's forward bending test and a manual scoliometer to measure rib hump asymmetry, which requires trained professionals and is prone to high costs and significant inter-rater variability. This project aims to overcome these limitations by developing an automated digital scoliometer using dynamic 3D back scans, enhancing both efficiency and comprehensiveness in scoliosis screening. Utilizing deep learning, we have automated the detection and localization of the spinous process line (SPL) within these scans, which is critical for accurate scoliometer placement. The Spinous Process Line (SPL) is a virtual line that aligns with the prominent protrusions (spinous processes) of the vertebrae, visible along the midline of the back, which is used as an anatomical reference in assessing spinal alignment. Our dataset included reconstructed forward bending sequences from 12 scoliosis patients, totaling 790 3D back scan frames. Depth maps generated from these back scans served as inputs for our model, with SPLs as the target outputs. We implemented a hybrid CNN-RNN architecture, integrating transfer learning models—ResNet50 and MobileNet—with a bidirectional LSTM to process the 3D data in a 2D framework. After 30 epochs of training, MobileNet outperformed ResNet50,

achieving a lower Euclidean distance loss (2.5 vs. 3.1 cm), indicating more precise SPL prediction. This advancement marks a significant step toward automating scoliosis screening, potentially reducing the need for manual palpation and making the process more accessible and consistent.

Vaiva Jakstaite

Home Institution: The University of Edinburgh

Host Institution: ETH Zurich

Project Area: Biotechnology

Project Supervisor(s): Prof. Dr. Alexander Harms

Shine Bright Like a Diamond: Engineering Bas34 to Express GFP Using CRISPR-Cas9

Conventional antibiotics are still widely used to treat bacterial infections. However, with the global spread of antimicrobial resistance, it is crucial to consider novel strategies for antimicrobial intervention. Bacteriophages (phages), viruses specifically infecting bacteria, can be used as an alternative for phage therapy. But a limiting factor for its effective implementation is our poor understanding of phage-host interactions at the molecular level since phage infections cannot be directly studied using light microscopy. To circumvent this problem, I am engineering a model bacteriophage Bas34 using CRISPR-Cas9 system to express a green fluorescent protein (GFP) in its genome so that bacterial infection can be tracked in real time. Out of four candidate guide RNAs (gRNAs), which will determine the locus of gfp insertion, the most efficient gRNA6 was selected. A plasmid expressing GFP was built to introduce gfp gene to Bas34 through homologous recombination. Engineered bacteriophages were counterselected using CRISPR-Cas9 system, and used to infect bacteria to trace the development of infection by detection of GFP signal during microscopy assay. Data obtained from these assays will be useful in better understanding phage-bacteria interactions at a molecular level, thus might help to enhance phage therapy protocols.

Yaren Karakoç

Home Institution: Bogazici University

Host Institution: ETH Zurich

Project Area: Ribosome Biogenesis

Project Supervisor(s): Stefanie Jonas

Ribosomes in the making: Interactome analysis of human 40S ribosome subunit biogenesis intermediates

Ribosomes are translations sites consisting of a small and large subunit. Ribosome biogenesis is the process in which ribosomes are generated from pre-ribosomal particles. Trans-acting factors which transiently bind to the precursor particle assist the biogenesis process at different stages. In our approach, mass spectrometry coupled to affinity purification has been implemented on fractionated cells to identify interactomes in different intermediates during the human small subunit maturation. To examine the entire process,

endogenously tagged 5 trans-acting factors were used as baits. My project consists of investigation of possible interaction between RPS3a and PARN suggested by mass spectrometry analysis and AlphaFold2 predictions and validation of expected trans-acting factors localization in the cell after tagging. Revealed by pull-down assays, RPS3a and PARN does not interact or only transiently and weakly interact. Validation of expected localization of trans-acting factors is shown in IF experiments.

University of Cambridge

Alexandra Ioana Ilie

Home Institution: Politehnica University of Bucharest

Host Institution: University of Cambridge

Project Area: Computational Biology/ Machine Learning

Project Supervisor(s): Professor Anton Enright

Detecting RNA modifications from Nanopore Signal Data

The detection and analysis of RNA modifications are crucial for understanding gene expression regulation and immune system interactions. Traditional next-generation sequencing (NGS) methods often fail to detect these modifications due to loss or blockage during reverse transcription. However, direct RNA sequencing on a nanopore device preserves these modifications, offering a more reliable method for analysis.

In this study, we developed a machine learning approach to analyse miRNAs from raw pico-amp current signals generated by Oxford Nanopore Technologies (ONT) sequencing. Our goal is to directly detect modifications in both native miRNAs and synthetic RNA constructs modified via click chemistry.

The nanopore sequencing process involves sample collection, library construction, and the passage of RNA through a nanopore, where changes in ionic current are detected and translated into nucleotide sequences. The raw signal is analysed using the second derivative to identify key components, such as adaptors, poly(A) regions and miRNA sequences. After collecting the miRNA, we align the sequences despite any temporal distortions. We then apply t-SNE for dimensionality reduction and proceed to cluster similar RNAs by converting alignment distances into a similarity matrix using a kernel.

Our deep learning model processes the RNA signal with a Bidirectional LSTM and attention mechanism, classifying the RNAs into two categories: unmodified RNAs and those with an Allyl group mutation. The model achieved an impressive 98.6% accuracy, demonstrating its effectiveness in classifying RNA sequences, even when signal differences are subtle.

Caitlin Turner

Home Institution: University of Leeds

Host Institution: University of Cambridge

Project Area: RNA Biology

Project Supervisor(s): Ian Brierley

Investigating The Role of RNA G-quadruplexes in Ribosomal Pausing

RNA G-quadruplexes (G4Qs) are folded motifs that are widespread and conserved in RNA virus genomes, but their role in virus biology remains elusive. Putative G4Qs have been identified in the coding regions of retroviruses and it was hypothesized that ribosomal pausing may contribute to a potential function in the regulation of protein production. To assess ribosome pausing, mRNA transcripts containing a thermodynamically very stable G4Q were translated in vitro in rabbit reticulocyte lysates (RRL). Aliquots of the translation mix were taken over a thirty-minute period and analysed by gel electrophoresis to detect translated proteins. Ribosome pausing would be evident from the accumulation of a smaller polypeptide. As ribosomes decode in triplets, three separate mRNAs were prepared with the G4Q placed in each of the three possible reading frames (phases) and pausing measured. Assays were also performed when the translation reactions were supplemented with pyridostatin (PDS), a G4Q stabilizing agent. It was found that the optimised G4Q did not efficiently pause the ribosome in vitro in any of the three phases. However, when the G4Q was stabilised with PDS, clear pausing was observed. These experiments reveal that the G4Q tested, unlike some other kinds of RNA structures, does not induce ribosome pausing in vitro. It is possible that the intrinsic ribosomal helicase can efficiently unwind G4Qs during translation, so pausing does not occur. Alternatively, the G4Qs are not forming in the RRL, perhaps because other RNA helicases present in the lysate are unwinding them. In this interpretation, the G4Q-stabilizing agent PDS would act to stabilise the G4Q, upon which it can act as a barrier to the ribosome. Further work is necessary to elucidate the role of G4Qs in virus replication. Immunofluorescence analysis of cells using an anti-G4Q antibody suggests this method may help confirm the presence of virus-encoded G4Qs.

Eoghan Leonard

Home Institution: Trinity College Dublin

Host Institution: University of Cambridge

Project Area: Biochemistry

Project Supervisor(s): Dr. Katherine Stott

Developing a Biophysical Toolkit for the study of Intrinsically Disordered Proteins

40% of the human proteome is made up of Intrinsically Disordered Proteins (IDPs), or proteins with Intrinsically Disordered Regions, which either wholly or partially lack a stable 3D structure, respectively. These proteins are becoming increasingly identifiable as disease causing agents, especially in neurodegenerative diseases. My project aims to develop a biophysical toolkit to better study these IDPs and gain insight into the Mass transport between the C-terminal domain of A Kinase Anchoring Protein 5 (AKAP5c) and Calcineurin (Cn). We wanted to create an experimental setup that moved away from the more ideal

system studies that have less crowded environments and no systematic diffusion, which are more commonly used. Thus, I made use of two label-free techniques to study my protein interactions. Firstly, Surface Plasmon Resonance via the Biacore T200 which features an alterable flow rate. Secondly, Biolayer Interferometry via the Octet Red96 which features an alterable vortex speed. The results I obtained from both of these techniques were transferred into EVILFIT processing software which uses a Bayesian fitting approach and makes no model assumptions related to the number and types of binding sites. This software produced a variety of graphs and plots that allowed us to better understand how useful each technique is for studying Mass transport in our IDP. We found that slower vortex speeds on the Octet tend to oversimplify the model so faster speeds are better for studying IDPs. Also, we found for the Biacore that slower flow speeds appear to increase Mass transport, presenting a good model for studying IDPs in cellular diffusion.

Iris Carvalho

Home Institution: University College Cork

Host Institution: University of Cambridge

Project Area: Cell Biology

Project Supervisor(s): Dr. Simon Bullock

Investigating Centrosomal Localisation of bicd2 mRNA in Mammalian Cells

A prevalent mechanism for establishing cell polarity involves trafficking of messenger ribonucleic acids (mRNAs) to specific locations in the cell. This process underpins many key cellular events, including embryonic axis formation, cell division and synaptic plasticity. However, the molecular mechanisms by which mRNA localisation is regulated in cells are poorly understood. My project investigated a potential role for post-translational modifications in regulating co-translational localisation of mRNAs to centrosomes of human cells. This process ensures accurate chromosome segregation by organizing the localization of proteins that regulate centrosome function. I specifically investigated the centrosomal localization of *Bicaudal D2 (bicd2)* mRNA, which encodes a protein essential for microtubule-based trafficking by the dynein motor. Recent findings show that Bicd2 protein is phosphorylated by Cyclin-dependent kinase-1 (CDK-1) during interphase and by Polo-like kinase-1 (PLK-1) during mitosis, but the impact of these events on mRNA localization remains unclear. Using in situ hybridisation and fluorescence imaging, I observed that *bicd2* mRNA localises to the centrosomes during interphase and late mitosis, with this enrichment lost at the onset of metaphase. Inhibiting CDK-1 activity using a chemical inhibitor led to a significant decrease in *bicd2* mRNA localisation to the centrosomes in interphase. I also discovered that a point mutation that abolishes the PLK-1 phosphosite in BicD2 causes a significant fraction of *bicd2* mRNA to remain at centrosomes during metaphase. Taken together, these results highlight a potential role for CDK-1 in localising *bicd2* mRNA in interphase, while subsequent phosphorylation of BicD2 protein by PLK-1 triggers dispersal of its mRNA at the onset of mitosis. In the longer term, results from this study may help uncover a broader role for post-translational modifications in RNA localisation, as well as pave the way for testing the function of mRNA localisation to the centrosome at different stages of the cell cycle.

Isobel Watts

Home Institution: University of York

Host Institution: University of Cambridge

Project Area: Plant Science

Project Supervisor(s): Edwige Moyroud

Genetic control of cell shape specification on petal surfaces and impact on pollinator perceptions

The colourful patterns on flower petals are essential for attracting pollinators. Hibiscus trionum flowers feature a striking bullseye pattern, with a central purple spot of flat, elongated, striated cells, and a distal white region of conical, smooth cells. To function as a communication device, this pattern must be exposed to pollinators when the flowers open. Consistently, it only develops on the adaxial (upper) petal epidermis. The mechanisms that limit this pattern to the adaxial epidermis remain unknown. In addition to genotyping CRISPR/Cas9 lines, I used microscopy and quantification (ImageJ), to analyse the distribution of cell features on the abaxial epidermis of H. trionum petals. I investigated the role(s) of the KNOX and LOB genes in shaping the two sides of the petal, and, to understand how pollinators perceive and respond to pattern variations, I conducted Bombus terrestris preference experiments using artificial flowers with different cell shapes. I found that the abaxial epidermis features elongated cells at the base and pavement cells at the top, with the largest cells located at the 1/3rd position from the base, mirroring the adaxial epidermis. I characterised knat6.1 loss-of-function mutant 1 and found that its petals display a smaller bullseye and more domed-shaped pavement cells than wild-type flowers. Additionally, I found that B. terrestris showed a significant preference for textured artificial flowers over smooth ones. While I identified the role of knat6.1 in cell shape specification and patterning and characterised loss-of-function LOB mutants, further research will explore the roles of additional KNAT and LOB genes to understand the gene regulatory network involved. Whether these genes are involved in specifying the cell shape or specifying the adaxial – abaxial epidermis need to be investigated. Finally, additional studies with homogenous cell shape patterns will help us clarify how bumblebees respond to texture patterns.

Jasper Krähe

Home Institution: Ruhr-Universität Bochum

Host Institution: University of Cambridge

Project Area: Chemical biology

Project Supervisor(s): Matthew Penner/Florian Hollfelder

Experimentally and computationally engineering a plant cell-wall degrading enzyme

The shift towards a sustainable bioeconomy facilitates an increasing demand for the biocatalytic harnessing of abundant biomass. However, engineering proteins for this task by random mutagenesis faces a vast sequence space of possible catalysts as well as an activity-stability trade-off, calling for a comprehensive approach. To properly investigate the activity-stability relationship we use an assay that decouples cellular abundance from activity and make use of microfluidic ultra-high throughput screening to cover up to 106 possible

variants. Complementary we employed molecular dynamics simulations to further investigate possible allosteric interactions that might show up in our screening campaign. We successfully implement and optimise an experimental workflow to create a library of more than 107 variants that is suitable for ultra-high throughput screening and can be used for the activity-stability decoupling assay. On the computational side we established a functional model to identify correlated structural dynamics of different domains in the protein. Ultimately, we aim to combine and synergize our assay and the screening campaign with the simulations to exhaustively navigate the sequence space and map and investigate possible allosteric interactions in our library and extrapolate from there to thoroughly engineer our enzyme target.

Jiahui Wu

Home Institution: University College London

Host Institution: University of Cambridge

Project Area: Molecular neuroscience

Project Supervisor(s): Jennifer Palmer and Prof. David Rubinsztein

The effects of autophagy inhibition on microglial functions

Introduction

- Autophagy is a conserved mechanism that degrades the damaged or superfluous cellular contents for recycling under nutritional stress.

- Microglia are glial cells functioning as resident innate immune cells in the central nervous system. They constantly sense the neural environment and clear debris to maintain homeostasis.

- Neurodegeneration is featured by progressive death of neurons. Impaired autophagy in both neurons and microglia can be associated with neurodegeneration.

Materials and methods

- We used BV2 microglia to simulate microglia, and employ techniques including western blots, immunofluorescence, time-lapse microscopy, cytokine assay, and flow cytometry.

Results and discussion

- From the results of western blots with Bafilomycin A1, we detected significant decrease in the proportion of phosphor-ATG14 and LC3-II with specific concentrations of ULK1 inhibitors ULK101 and MRT68921.

- Preliminary data shows distinct profile for both cytokine up-regulation and down-regulation upon autophagy inhibition in BV2 microglia.

- The phagocytosis profile affected by autophagy inhibition in BV2 microglia is inconsistent and it needs further validation. Inconsistency between replicates may be due to pipetting error for small-volume samples. More accurate technique for quantification is required, such as flow cytometry.

Maurice Leung

Home Institution: University of Oxford

Host Institution: University of Cambridge

Project Area: Cancer Genomics

Project Supervisor(s): Hui Zhao

Comparing Library Preparation Methods for FFPE Tumor DNA: A New Avenue for Tumor-Informed Liquid Biopsy

Liquid biopsy and circulating tumor DNA (ctDNA) analysis is an emerging field in cancer diagnostics. Tumor-informed ctDNA assays offer greater sensitivity and specificity than tumor-naïve methods, emphasizing the importance of primary tumor sequencing. Formalin-fixed, paraffin-embedded (FFPE) tissue is an ideal medium for this purpose given its ease of storage and the vast historical specimens available. However, there remain various bottlenecks in FFPE tissue sequencing, requiring both computational and wet-lab solutions. This study investigates how changes in library preparation methods can improve the sequencing of FFPE tumor DNA. FFPE tumor tissue from two lung squamous cell carcinoma patients in the MISIL1 cohort were sequenced using NovaSeq 6000 following various library preparation methods; methods with/without FFPE repair, with enzymatic fragmentation vs. Covaris sonication, and with different library preparation kits. Bioinformatic tools were subsequently used to conduct secondary analysis of the sequencing data, revealing key differences in metrics like coverage, soft clipping, insert sizes, mismatch rates, and mutation calling. Notably, enzymatic fragmentation reduced soft-clipping, lengthened insert sizes, and was linked to greater coverage, compared to Covaris sonication. In addition, the Agilent XT HS2 kit was linked to flatter mismatch profiles and FFPE repair reduced the presence of sequencing artifacts. These results indicate that incorporating FFPE repair, enzymatic fragmentation, and the Agilent XT HS2 kit into the library preparation process optimizes sequencing outcomes. This optimized sequencing protocol should be coupled with computational solutions to leverage the translational potential of FFPE tumor tissue, ultimately enhancing the accuracy of cancer diagnostics and enabling more personalized treatment approaches.

Răzvan Mihail Ciurescu

Home Institution: École Polytechnique

Host Institution: University of Cambridge

Project Area: Psychiatry/Bioinformatics

Project Supervisor(s): Sarah E. Morgan

Mind your MIND: Linking structural and functional brain connectivities through statistical analysis

The brain can be viewed as an interconnected graph and a key focus of Network Neuroscience is studying the clinical implications of the way different brain regions - the nodes - interact. The main modalities which quantify how regions are connected are structural and functional connectivities; and (Sebenius et al., Nature Neuroscience 2023) recently proposed a new approach (MIND) to estimate the former. The aim of this project was to study the link between MIND brain networks and functional connectivity (FC)

networks from a statistical perspective, and determine whether MIND brain networks can be used to predict FC networks. The methods used included the fingerprinting approach – a mathematical tool meant for quantifying if an individual's MIND network correlate better with their own FC network than with the FC networks of other subjects (Finn et al., Nature Neuroscience 2015) as well as using a multi output regression algorithm to predict the brain structure from the brain function. The connectivity matrices, as well as the characteristics of individuals (sex, age, etc.) come from two datasets: the Human Connectome Project (HCP) and the UK BioBank (UKBB). Although the mean statistical correlation between MIND and FC networks per region was weak, reaching at most r=0.30 for the UKBB dataset, the percentile approach showed a 70th-percentile median value for the UKBB - which proves the existence of an underlying relation between one's MIND and FC networks - compared to a 50th-percentile median value for the HCP. Moreover, functional networks could be predicted via a machine learning regression algorithm from the UKBB MIND networks with a 66% accuracy. These results point to future possibilities of generating the FC networks from MIND networks, leading to a deeper understanding of the relation between structure and function in the brain.

Veronia Nasralla

Home Institution: Utrecht University

Host Institution: University of Cambridge

Project Area: Cancer Biology

Project Supervisor(s): Dr. Daniel Muñoz Espín

Exploring Senescence in the Tumour Microenvironment of a Chemically Induced Lung Cancer Model

Introduction - Lung cancer remains the leading cause of cancer-related mortality worldwide, with its prognosis remaining poor despite advancements in treatment. Cellular senescence, a state of stable cell cycle arrest triggered by different stressors, has recently been recognised as an emerging hallmark of cancer. While senescence initially acts as a tumour-suppressive mechanism, chronic senescence can activate the senescence-associated secretory phenotype (SASP), paradoxically promoting tumourigenesis and cancer progression. However, the study of senescence in different cancer types, such as lung cancer, is hindered by the lack of robust models. The aim of this project was to validate a chemically-induced lung cancer model to detect senescent cells in the tumour micro-environment of lung cancer.

Materials & Methods - N-methyl-N-nitrosourea (MNU) was used to induce lung cancer in mice, assessed by histological and immunohistochemical analysis in p16-FDR-mCherry mice. Additionally, the detection of senescent cells (mCherry+, DAPI-) in these mice was assessed using flow cytometry.

Results - Genetically modified mice carrying the p16/FDR cassette developed visible tumours following MNU treatment, which were further validated through histological and immunohistochemical analyses. Moreover, flow cytometry analysis of lung tissue showed that all mice displayed mCherry+ cells, indicating the presence of senescent cells in this lung cancer model.

Discussion/Conclusion - Overall, our findings demonstrate that MNU treatment successfully induce lung cancer in mice, and that the p16/FDR-mCherry mouse model could be an

effective tool for studying senescence in the context of lung cancer. Therefore, this model could pave the way in understanding the tumourigenesis of lung cancer, offering new avenues for developing future treatments, and ultimately helping millions of patients.