THE HUMAN FRONTIER SCIENCE PROGRAM
ANNOUNCES ITS

2024 Fellowship Awardees






35 years

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The Human Frontier Science Program (HFSP) is unique in supporting international collaboration to undertake innovative, risky, basic research at the frontiers of the life sciences. Special emphasis is given to the support and training of independent young investigators, beginning at the postdoctoral level.

The Program is implemented by the International Human Frontier Science Program Organization (HFSPO), supported financially by Australia, Canada, the European Commission, France, Germany, India, Israel, Italy, Japan, New Zealand, Norway, the Republic of Korea, Singapore, South Africa, Switzerland, the United Kingdom of Great Britian and Northern Ireland, and the United States of America.

Since, 1990, more than 8,500 researchers from more than 70 countries have been supported. Of these, 29 HFSO awardees have gone on to receive the Nobel Prize.

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About HFSP's 2024 Fellowships
Message from the Director of Fellowships:

Barbara Pauly,
Director HFSP
Fellowship Program

This year's HFSP Fellowship Award Winners are among the world's most creative and courageous postdoctoral investigators taking brave steps to broaden their skills and their horizons early in their careers.

For 2024, HFSP has chosen to support 59 Fellowships to post-doctoral researchers representing 28 nations, some of whom have dual citizenship, which is noted. Their projects span 10 areas of research that range in focus from the most complex neuroscience to cross-disciplinary investigations that seek to understand evolution across populations. Fellows work in the laboratory of a host scientist in a country that is different from where their PhD was conferred. In some cases, the fellow will have more than one supervisor; thank you to the 68 hosts who are accepting HFSP fellows for the 2024 cycle. Fellowships last for three years and on average, each awardee will receive $200,000 USD for the full fellowship.

In this volume, our first HFSP Fellowship Awards Booklet, you will find the abstracts that impressed our review committee. They felt these projects offered the promise of truly expanding the frontiers of life science research and may well lead to unimaginable findings! HFSP offers Long-Term Fellowships and Cross-Disciplinary Fellowships.

Long-Term Fellowships are for applicants with a PhD in a biological topic who want to embark on a novel frontier project focusing on the life sciences.

Cross-Disciplinary Fellowships are for applicants who hold a PhD in a non-biological discipline (e.g., physics, chemistry, mathematics, engineering, or computer sciences) and who have no prior training or research experience in the life sciences, but want to work on a novel frontier project in biology.

All HFSP Fellowships provide an annual living allowance as well as a research and travel allowance. In addition, child, parental leave and relocation allowances are provided where appropriate. Applicants from a country that is not a Member of HFSPO must hold their fellowship in a HFSPO Member country. To take a look at the Fellowships we have funded in the past, please visit our website at www.hfsp.org.

Congratulations are in order to all of our winners as they begin their fellowships. Please know, we're all looking forward to see what you discover!

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01 Molecular and Structural Biology

Protein Dynamics and Function in the Evolutionary History of the CO2 Fixing Enzyme Rubisco

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Traditionally, protein function is thought to be determined solely by structure. However, biomolecules are not always confined to one structural conformation, and it has long been conjectured that motions between different conformations can explain differences in function, where there are no differences in ground state structures. Advances in Nuclear Magnetic Resonance spectroscopy (NMR) allow such motions to be probed across many different time-scales, even in complexes up to 1 MDa using selective isotopic labelling schemes. The promise of this capability has not yet been fully realized, as it is generally impossible to causally link particular motions to specific differences in protein function.

I propose to make this connection by retracing how protein function and dynamics changed in concert across evolutionary history. To establish this paradigm, I will use the socio-economically relevant model system Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco, which is responsible for almost all current carbon fixation. During its history and evolution, Rubisco's catalytic mechanism has undergone important changes almost certainly caused by differences in its motions and dynamics.

Firstly, Rubisco has an undesired side-reaction with oxygen, whose product has to be detoxified in a set of reactions that lead to agriculturally significant losses of carbon and nitrogen. Plant Rubiscos distinguish between CO2 and O2 better than their bacterial precursors, but how they accomplish this remains unknown; both kinds of Rubisco have identical active sites in available crystal structures. Secondly, plant Rubiscos depend on a specific ATPase (a so-called activase) to periodically remove sugar phosphates other than its native substrate from its active site. This kind of 'metabolic repair' is unnecessary in older forms of Rubisco, where the molecules diffuse out of their active sites unaided.

I hypothesize that these important differences in function are ultimately caused by differences in protein motions. I will prove this causal link by first retracing the evolution of these functional changes using ancestral sequence reconstruction and functional characterization of resurrected enzymes (both established in the Hochberg lab). I will then use NMR techniques that probe motions from pico- to micro-second dynamics in large proteins that I used and developed during my doctoral work to test which dynamical changes are associated with historical substitutions that led to changes in Rubisco's catalytic properties.

This work will create a new paradigm for how the dynamical underpinnings of protein function can be unraveled and reveal the biophysical basis for geochemically significant changes in the function of one of life's most central enzymes, which may ultimately help rationally improve Rubisco. It will also be a first step in my long-term ambition to illuminate causal links between protein dynamics and function.

Illuminating Orphan GPCR Signaling by Novel Biochemical Approaches

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G protein-coupled receptors (GPCRs) are important for cells to sense their external environment. Studies on their structure, pharmacology, and so on have led to successful therapies of GPCR-related diseases. However, there are still hundreds of «orphan» GPCRs, whose function remain unknown. Revealing the function and ligands of orphan GPCRs is called “deorphanization”. Deorphanization of these receptors could have significant implications for human physiology and disease treatment, but efforts to find their activating molecules have slowed down over the past decade. The lack of an activator makes it difficult to study downstream signaling pathways and to develop assays for probe molecule discovery of orphan GPCRs. My research aims to develop new approaches to illuminate the function of orphan GPCRs, particularly those expressed in the central nervous system.

To overcome these challenges, I propose a new approach to purify orphan GPCRs and use them as ”bait” to find endogenous hormones or surrogate activators for orphan GPCRs by combining such disciplines as biochemistry, native mass spectrometry, antibody development, and structural biology. As a prototype of screening of orphan GPCR activators, I chose GPR85, the most conserved GPCR in all vertebrates. Knock out of GPR85 leads to a 20% increase in brain mass, while overexpression studies implicate it as a negative regulator of hippocampal neurogenesis and spatial learning. But the lack of ligand of GPR85 hinders understanding of the precise function and signaling of GPR85.

My research has two aims. First, identify endogenous ligands for GPR85 using mass spectrometry and NMR techniques. To accomplish this, I will use extracts from the hippocampus to identify other molecules that bind tightly to GPR85. The identified molecules will be used to determine the cellular signaling pathways modulated by GPR85. Second, use combinatorial antibody libraries to find new surrogate ligands for GPR85. The approach will identify molecules that bind to GPR85 in a specific conformation and have the potential to induce or inhibit GPR85-mediated signaling. The candidate molecules will be screened for activity against a panel of cellular signaling pathways.

Orphan GPCRs represent a huge opportunity for drug discovery. A new approach could shed light on the function of these mysterious receptors and pave the way for similar approaches to tackle other orphan receptors. The ultimate goal is to illuminate the function of one of the most intriguing orphan GPCRs in the genome and pave the way for similar approaches to numerous other orphan receptors. This approach could lead to the discovery of new therapies for recalcitrant diseases.

Is Quantum Coherence Important in Coupling the Antenna System to the Photosystem in Cryptophytes?

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Cryptophytes are single-celled, aquatic algae faced with the challenge of limited photons, which requires them to have a very efficient light-harvesting process. Cryptophytes possess an antenna comprised of phycobiliproteins (PBPs) that increases the photon absorption area and the accessible spectral range of the incoming photons. The incoming photon creates an electron-hole pair called an exciton, which needs to move from the antenna to the photosystem quickly and efficiently. Evidence of quantum beats suggests that this process of moving the exciton via several PBPs employs quantum coherence. However, the most efficient way for the exciton transferal is a mixture of coherent and incoherent transfer steps. The intra-protein interactions are probably based on coherent excitation transmission while the transfer process between PBPs is incoherent.

We hypothesize that the PBP linking the antenna with the photosystem operates coherently. Recently, it has been discovered that PBPs come in two different quaternary structures: the open and the closed forms. The closed form exhibits coherent beats while open form does not. The main goal of this project is to determine the role of quantum coherence in the light harvesting systems of cryptophytes.

We plan to investigate the quantum mechanical nature of the linking protein that connects the antenna proteins with the photosystem in cryptophytes. To achieve this objective, we will employ a combination of experimental and computational methods. First, we plan to determine the single particle structure of this linking protein between the photosystem and the antenna proteins using state-of-the-art in situ cryo-electron microscopy (cryo-EM). In the next step, we would build a molecular dynamics simulation based on this newly found structure and perform quantum mechanics/molecular mechanics (QM/MM) computations to evaluate possible exciton transfer pathways.

By investigating the quantum coherence in the linking protein between the antenna and the photosystem, our research project aims to uncover the missing piece in the interplay of coherent and incoherent exciton transfer. These kinds of linking proteins between antenna structures and the photosystems are not unique to cryptophytes, for example the FMO protein in green sulfur bacteria is also thought to employ quantum coherence. We hope to uncover the reason for this apparently general requirement for linking proteins to function in a coherent way and discover how the linking protein is able to uphold the long-lived quantum coherence against the thermal motion of its surroundings. Understanding the quantum biological nature of the linking protein holds immense promise for unlocking the quantum secrets of cryptophytes and shedding light on the fascinating interplay of coherent and incoherent exciton transfer in several species, with potential applications in quantum technologies and bio-inspired design of efficient energy harvesting systems.

02 Organelles, Cells, Tissues and Organs

Structural Basis of the Human Spliceosome Quality Control

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Splicing of newly synthesized precursor messenger RNAs (pre-mRNAs) is essential for eukaryotic gene expression and involves the removal of introns and subsequent ligation of exons to produce a mature protein-coding mRNA. In humans, splicing is carried out by over 150 proteins and five small nuclear RNAs that assemble into a dynamic multi-megadalton complex called the spliceosome. A single round of splicing requires an exceedingly complex series of conformational and compositional transitions within the spliceosome. With so much room for error, how is aberrant splicing prevented? Recent evidence suggests that mechanisms for spliceosome quality control (QC) exist, but their molecular basis remains unknown.

QC mechanisms have evolved at every step of gene expression to ensure the faithful execution of genetic instructions. By analogy with the well-studied QC mechanisms in protein translation, I hypothesize that the spliceosome can form aberrant conformational and compositional states that, if unresolved, may result in faulty mRNAs and a depleted pool of spliceosome components, impeding gene expression. Thus, while canonical splicing events are increasingly well understood, elucidating how aberrant off-pathway spliceosomes form, how they are recognized and dealt with represents a major knowledge gap. With this project, I aim to expand our mechanistic understanding of pre-mRNA splicing beyond canonical events by elucidating the molecular basis of human spliceosome QC using a combination of structural and functional approaches. Understanding spliceosome QC may shed light on the molecular basis of diseases linked to aberrant splicing and may also reveal the role of QC factors in alternative splicing.

Combining Spatial-omics with AI to Model Developmental Gene Regulation in Space and Time

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A longstanding goal is to understand how the genome encodes the differentiation trajectories from fertilized egg to the large complexity of cell types in the adult. The challenge is to exhaustively sample all possible cellular states to capture all emergence of the genome. New image-based spatial transcriptomics techniques now have the capability to rapidly measure the transcriptomes of all cells of an entire small organism such as the fruit fly Drosophila melanogaster.

This project proposes to (1) measure and reconstruct all differentiation trajectories of D. melanogaster in space and time from egg to adult. These lineages are then (2) integrated with single-cell chromatin accessibility data and AI models for Gene Regulatory Network (GRN) inference to mechanistically understand how enhancers and transcription factors (TF) orchestrate cellular differentiation and identity. Lastly, (3) GRN AI models will be extended to directly predict spatiotemporal gene expression from the genome sequence.

The link between the genome sequence and spatio-temporal gene expression can be measured and learned. The 3D single-cell spatial transcriptomes of the 10-day Drosophila developmental time course will be measured with a 4-hour interval. We will measure 1,600 genes, including all 708 TFs, canonical patterning genes and cell type markers. The cellular states will be identified and linked between timepoints to reconstruct the differentiation trajectories. This challenge is made easier by leveraging the spatial locations of progenitors and putative progeny that greatly constrains the number of possible links. The large gene panel will facilitate dataset integration with matching fly cell atlases of transcriptome and chromatin accessibility generated by the host lab, so that knowledge from other modalities can be transferred to the 3D fly development model.

In particular, inferred GRNs from these data will be aligned so that enhancers and TFs important for lineage decisions can be identified to understand trajectories. With the extensive genomics toolbox of Drosophila, the identified actors will be validated in vivo. The current AI tools of the host lab can predict the downstream gene targets of TFs based on their expression level, the TF binding sites within genomic enhancers, and the accessibility activity of the enhancers. Using the integrated spatial data, these models will be extended to also predict where genes will be expressed in the body, and when in development these targets turn on.

The project will pioneer whole body biology where a complex measurement is taken of virtually all possible healthy cell states of an organism, so that they can be linked into developmental trajectories without gaps. Integrating these with mechanistic GRN information will identify the actors driving the lineage choices, so that we can understand and possibly manipulate the language of the genome.

Unraveling the Molecular Mechanisms of Thyroglobulin Endocytosis Mediated by the R2 Receptor

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Thyroid hormones are essential for development and metabolism in all vertebrates. Thyroid hormone imbalance severely compromises the well-being of about 10% of the world population and represents a yearly financial burden of over several billion dollars for the health system in a country like the United States of America.

However, the molecular mechanism behind their regulation is far from being understood, as is their tuning in vivo. Thyroid hormones are synthesized via iodination of their protein precursor thyroglobulin within the extracellular lumen of thyroid follicles, then released into the bloodstream to reach target tissues in the organism (e.g., brain, muscles, liver). Thyroglobulin endocytosis is a key step for thyroid hormone release and a membrane protein receptor (TGR) has been shown to mainly promote thyroglobulin internalization from the extracellular lumen. How thyroglobulin recruitment and internaliz-ation machinery works in different cellular conditions remains an outstanding question in the field.

We propose to fill this knowledge gap by unraveling the mechanistic aspects of thyroglobulin endocytosis by TGR via an integrative structural biology approach. First, using single particle cryo-electron microscopy (cryo-EM), we aim to elucidate the first structure of human thyroglobulin receptor in isolation and in complex with thyroglobulin, identifying the determinants of iodinated thyroglobulin recognition by TGR, mapping the effects of disease mutations. Secondly, using a combination of proteomics, gene editing, and cellular endocytosis assays, we aim to dissect the complete machinery of the thyroglobulin internalization complex. Finally, we seek to visualize it in situ, recapitulating the internalization process directly in cells.

We expect to decipher the rules of thyroglobulin endocytosis in the thyroid, opening a new front for targeted thyroid hormone control in organisms. Since TGR is also expressed in many other tissues and mediates the transport of other organ-specific cargoes this study will have ample application to understand key trafficking routes in other tissues.

Targeted Epigenome Editing Using Induced Proximity-based Small Molecules

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Epigenetic modifications modulate gene expression and suppression, which strongly affect embryonic development, cell differentiation, and many genetic diseases, including cancer. While DNA methylation has been widely studied as one of the important epigenetic modifications, there is no universal tool to artificially edit DNA methylation to specific loci. Recent work has demonstrated that a dead Cas9 (dCas9) fused to a catalytic domain of DNA methyltransferases (DNMTs) successfully induced DNA methylation in loci-specific manner using guide RNA (Nunez J. K. et al. Cell, 2021). These tools are powerful to study epigenetics, however, it faces packaging and delivery challenges because of their huge molecular weight.

This project aims to develop a small-molecule-based DNA methylation platform as a tool for selective epigenome editing at specific loci. This platform will enable us to study the effect of DNA methylation in stem cells and patient-derived cells with high modulating efficacy because small molecule has good cell permeability. Furthermore, it could also be applied to study tissue-specific effects of epigenomics in vivo.

We will use covalent chemoproteomic platforms to find small-molecule covalent recruiters targeting allosteric non-functional sites within DNMTs as well as within demethylases (TETs). Upon identifying suitable recruiters, which retain the catalytic activities of their respective enzymes, we will take two orthogonal approaches. First, we will use a small-molecule-based approach to conjugate our DNMT or TET recruiters to small molecule ligands for transcription-related factors, such as p300, BRD4, and EZH2, or onto G-quadruplex targeting ligands previously reported in literature. Second, we will pursue a bioconjugation approach, in which our DNMT or TET recruiters are attached to dCas9 with guide RNA or pyrrole-imidazole polyamide (PIP), which could recognize target DNA in a sequence-specific manner.

We hypothesize that these conjugates will recruit DNMTs or TETs to actively transcribed or transcriptionally repressed genetic loci to methylate or demethylate these sites to suppress or activate transcription of these sites respectively. This approach could be useful as a chemical tool for modulating transcription or even a therapeutic approach for diseases such as cancer, in which cancer cells often show aberrant activation of oncogenic loci and suppression of tumor-suppressor loci. Our second concept will enable more control of methylation and transcriptional regulation at specific genetic loci. It could be useful for studying cell development or differentiation by changing the translation state of specific loci.

Our tools will elucidate which DNA methylation or demethylation is responsible for embryonic failure, and which loci is relevant for oncogenesis or cell differentiation. We expect this approach could also be applied to other editing strategies, such as histone acetylation or RNA epitranscriptomics.

Reconstructing the Ancestral Architecture of Animal Cells

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Choanoflagellates, the closest living relatives of animals, have emerged as a powerful model system to study the origin of animal morphogenesis and multicellularity. These aquatic microbes share a significant portion of critical animal genes and cell architectures, such as the apical collar complex (acc), comprised of a central flagellum surrounded by a ring of microvilli. Many choanoflagellates have transient multicellular life stages, and *Choanoeca flexa*, a recently discovered species, is capable of emergent collective behavior. Sheet colonies of *C. flexa* are formed by intercellular links at the microvilli, and can switch between swimming and feeding conformations via collective apical constriction that alters collar geometry, reminiscent of mechanisms of animal tissue morphogenesis. Despite their evolutionary and functional relevance, little is known about the structure, patterning and remodeling of the acc, and the nature of intermicrovillar links in *C. flexa* is entirely unknown.

I hypothesize that the mechanisms that govern cell and tissue architecture in animals were largely inherited from their single-celled ancestors and can still be identified in choanoflagellates. Moreover, I hypothesize that common principles underlie acc formation, intermicrovillar adhesion and collective contractility in choanoflagellates and animals. My aim is to elucidate the processes that control choanoflagellate cell and colony shape by investigating acc ultrastructure and formation and characterizing the intermicrovillar links and cellular basis of colony inversion in *C. flexa* respectively.

I will expand the current toolbox of choanoflagellate research (predominantly comparative genomics, transcriptomics, and more recently, functional genetics) with state-of-the-art electron microscopy to characterize high-resolution and structural aspects of different choanoflagellate life stages and cell-cell interactions. I will use cryo-electron tomography for the ultrastructural characterization of the acc in Salpingoeca rosetta and *C. flexa*. Next, I will use sub-tomogram averaging to resolve the molecular composition of intermicrovillar links, within and between cells, to identify conserved and unique components that mediate the intercellular contact. Additionally, I will reconstruct whole-cell architectures of *C. flexa* cells and colonies in both conformations using focused ion beam scanning electron microscopy and quantify subcellular arrangements that mediate the switch. To gain insight into acc formation, I will use live-cell confocal microscopy to image cells during induced acc regeneration. Finally, I will perform drug screens and use high-throughput automated imaging to dissect the molecular pathways involved in acc formation.

This work will provide insights into the molecular and cellular regulation of the collar complex in choanoflagellates and provide a comparative framework for the origin of cell organization in metazoans.

Decoding Mechano-epigenetic Memories in Tissue Regeneration

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Tissue regeneration is essential to life and requires efficient re-establishment of functional tissue architecture and its mechanical properties. However, as an evolutionary trade-off for terrestrial life, skin wounds in large mammals typically heal by fibrosis, which can evolve into dysfunctional scars. Strategies to promote regenerative, rather than maladaptive fibrotic, healing have been limited, in part due to incomplete understanding of the fundamental mechanisms that derail adequate healing response. There is an emerging appreciation that tissue-resident cells accumulate “epigenetic memories” of past “stressful” experiences. Persistent scarring profoundly alters the mechanical cues of cellular niches, namely the stiffness, and viscoelastic properties. While it is known that mechanical signals are critical for scarring behavior, the notion of epigenetic memories triggered by mechanical stress and impacting mechanochemical feedback loops in tissue regeneration has not been addressed.

It remains unknown whether mechanical memories can be bestowed in cells in vivo, what are the molecular mechanisms, and finally what are the pathophysiological consequences of such effects? Here, I will investigate how various skin stem cell populations respond to viscoelastic mechanical cues in scarring and whether they have capacity to learn from past experiences by forming mechano-epigenetic memories to impact future healing outcomes.

This project will involving mapping the mechano-epigenomic landscape in scarring using integrative atomic force microscopy and spatial ATAC-seq and CUT&Tag. I will then elucidate how mechanical memories are induced and recalled in mouse models of skin scarring. Likewise, I will mechanistically dissect how mechano-memory reshapes healing outcomes using in utero lentivirus overexpression of identified mechanical memory peaks.

I hypothesize that altered niche viscoelasticity in fibrotic scarring triggers epigenetic re-wiring of cells, and that skin cells in mechanically stressed niches acquire ‘geographically’ distributed mechanical memories that can be decoupled from inflammatory memories. Further, I suspect skin cells imprint acquired mechano-memory in their nuclear architecture and surrounding matrix, and that mechanical memory is sufficient to induce maladaptive scarring and accentuate subsequent healing responses.

I will use an interdisciplinary approach combining in vivo mouse models, state-of-the-art hydrogel engineering and biophysical methods with data-driven bulk and spatial epigenomics technologies. This combines my expertise in bioengineering and clinical medicine with the expertise of the host lab in stem cell biology and mechanoregulation of chromatin architecture.

Visualizing Specific Lipid Processing and Uncovering Molecular Mechanisms Using Gut Organoids

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Lipids are one of the most essential nutrients for biological activity and play a variety of roles, including energy storage, construction of cell membranes, and intracellular signal transduction in developmental biology and cancer. There are more than 1,000 different types of lipids in a typical eukaryotic cell. However, it is still not well known how the structural diversity of lipids functions differently in vivo context. Although lipid research has made progress in the identification of lipid species using mass spectrometry, it falls behind protein research, especially in terms of imaging. Unlike proteins, successfully observing lipid species under light microscopy at high resolution using gene editing or chemical reactions – without perturbing their structures and functions – has not been achieved.

Recently, a technique called bifunctional lipid probes has been developed using photo-activation and click chemistry techniques. This technique enables us to observe the localization of near-native lipid species in cells with high resolution fluorescence microscopy. I aim to investigate the biological significance of the structural diversity of lipids in terms of how they function and are involved in biological contexts by combining a novel lipid visualization technique with super-resolution microscopy observations and mass spectrometry. To demonstrate the feasibility of this approach in monitoring lipid dynamics, I will apply the visualization system to the small intestinal organoids model. The small intestine is one of the major lipid processing organs such as uptake, transport, subcellular conversion, and secretion (lipid flux). This organoid model exhibits intracellular transportation of lipids and offers convenient imaging accessibility. I hypothesize that lipid structure (e.g., head group, chain length, and saturation) determines trafficking routes and kinetics within intestinal cells. I will endeavor to reveal the spatiotemporal dynamics and hidden biological functions of structurally diverse lipids with unprecedented resolution. This project will provide a novel platform for the analysis of lipid flux at the intercellular and tissue level and an important resource to reveal the function of specific lipids in homeostasis and disease.

After this proof-of-concept experiment, I will apply the imaging system to the mouse primordial germ cell (PGC) culture system, where I established an imaging system. PGC development has unique and interesting dynamics of lipids. For example, PGCs have transiently conspicuous lipid droplets during the migration to future gonads, however, their function is still unknown. Thus, this future study will provide novel insights into lipid function during germ cell development.

Elucidating the Role of R-loops in Hematopoiesis and Bone Marrow Niche Remodeling in Aging

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Hematopoiesis is the process by which all cells in the blood are produced and contributes to organismal health. During aging the hematopoietic system undergoes several changes, including a differentiation skew of the hematopoietic stem cells towards myeloid immune cells, which leads to a decrease in lymphoid immune cells. Thus, aging is a major risk factor for several blood malignancies. At the same time, the mesenchymal stroma cells (MSCs) that are crucial for supporting hematopoiesis become skewed towards adipogenesis with age. However, the molecular mechanisms that contribute to this age-associated decline in the hematopoietic system remain understudied.

R-loops, or DNA:RNA hybrids, are co-transcriptional molecules that when misprocessed lead to transcriptional dysregulation, DNA damage, and genome instability. Due to these detrimental events, normal cells efficiently process and remove R-loops. A study that I recently conducted showed that aging neurons display defects in R-loop metabolism, which in turn has a detrimental effect on neuronal function, and concomitant changes to the transcriptional landscape. In addition, the host lab has identified key R-loop-related enzymes downregulated upon aging in bone-marrow-derived mesenchymal stroma cells (MSCs). Altogether, these observations posit R-loops as a potential mechanism that contributes to the loss of hematopoiesis support during aging and disease.

In this project, I will connect for the first time R-loop metabolism and aging to changes in the hematopoietic stem cell niche. My central hypothesis is that the age-associated changes of R-loop metabolism in the MSCs lead to transcriptional dysregulation of gene regulatory networks associated with differentiation potential, which in turn remodel the bone marrow niche.

To test this hypothesis, we will determine the specific molecular pathways that are regulated by R-loops in MSCs and establish whether modulation of R-loops can change the differentiation potential of MSCs and remodel the bone marrow niche.

This project will involve two goals. We will profile R-loops and R-loop binding proteins in MSCs from young and old mice and human samples. Data integration with RNA-seq will allow us to identify R-loop regulated genes and establish R-loop associated molecular pathways. We will test how modulation of R-loop metabolism contributes to differentiation potential of MSCs using siRNA-mediated depletion of prioritized R-loop binding protein targets, followed by single cell (sc) RNA-seq and scATAC-seq of the bone marrow. For this, we will co-culture siRNA-loaded MSCs with hematopoietic stem/progenitor cells (HSPCs) followed by single cell multi-omic assays as a phenotypic readout of differentiation. We hope to elucidate how R-loops maintain MSCs homeostasis during aging and how R-loop metabolism contributes to the bone marrow niche remodeling.

Molecular Mechanisms and Tissue Mechanics Driving Spinal Cord Regeneration

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The spinal cord sustains animal locomotion and bridges neuronal connectivity from the brain across the body. While vertebrates such as fish can fully regenerate their spinal cord after injury, humans suffer permanent paralysis. Studying the coordination of tissue fluidization, cellular dynamics, and extracellular cues can provide novel insights into mechanisms of spinal cord regeneration. I propose to study spinal cord regeneration in zebrafish larvae using advanced 3D live imaging coupled with optogenetics and quantitative image analysis from the tissue to the subcellular scale. In addition, I will use my expertise in organelle dynamics to identify molecular mechanisms of tissue phase transition and cell migration during regeneration.

My project will focus on four main areas. First, I will map tissue mechanics and cell dynamics during spinal cord regeneration. I will use advanced 3D live imaging to segment and track glial and neural cells over time to measure cell migration, morphology, topology changes and axon growth. I will also measure rostro-caudal yield stress variations of the injured tissue, using in-plane μ-indentation for simultaneous live imaging and mechanical measurement. I will perturb cell and tissue mechanics by modulating Rho/Rho-kinase-induced actomyosin contractility with OptoGEF/OptoGAP and quantify its effect on regeneration rate. Second, I will unveil the role of directional secretion in spinal cord regeneration. I will use novel lines to build a correlative map of ER-Golgi localization, protein secretion dynamics and tissue stress. I will then perturb directional secretion with pharmacological inhibitors, and photoactivable-Rac1-induced ER-Golgi reorientation, and measure the effect on tissue fluidity and regeneration rate. Third, I will investigate the role of pro-regenerative extracellular cues in controlling tissue mechanics and directional secretion. I will test to determine whether growth factors, critical for regeneration, form rostro-caudal gradients and how they affect tissue mechanics and subcellular dynamics. I will use knock-out lines and pharmacological techniques to alter these gradients in vivo while examining tissue fluidity and subcellular dynamics. Finally, using OptoGEF/OptoGAP system under these conditions, I will investigate whether tissue mechanics drive spinal cord regeneration.

This project will seek to uncover previously unknown biochemical and mechanical crosstalk that may be driving spinal cord regeneration. This knowledge has the potential to contribute to the development of new strategies to boost regeneration in mammals.

Molecular Mechanism of Sister Chromatid Resolution in Replicated Human Chromosomes

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Supervised by Daniel Gerlich

Faithful transmission of genetic information across cell progeny requires disentanglement and separation of extremely long DNA molecules after replication. Sister DNA molecules are first resolved and packaged into separate bodies by condensin-mediated DNA loop extrusion and DNA strand crossing mediated by topoisomerase II (TOP2A in vertebrates), followed by long-range movement mediated by the mitotic spindle. While spindle mechanics are understood in great detail, it has remained unclear how condensin and TOP2A cooperate in sister chromatid resolution. While TOP2A resolves inter-chromatid entanglements, it also induces intra-chromatid entanglements to enhance the mechanical stability of chromosomes during mitosis. How these two activities are coupled with condensin-mediated DNA looping is not known, as no method can simultaneously detect condensin and TOP2A activities in the same cell to assess sister chromatid topologies.

In this project, I aim to develop a new method for genome-wide mapping of TOP2A activities within and across sister chromatids along with DNA contacts mediated by condensin at the single-cell level. To achieve this, I will combine my chemical ligation-based DNA barcoding technology to retain nuclear and spatial information of genomic DNA fragments and antibody-coupled DNA tags with the host lab's sister-chromatid-specific nascent DNA labeling strategy using a thymidine analogue. After sequencing the barcoded library, the barcodes enable the reconstruction of genomic sites undergoing strand passing by TOP2A and loop extrusion by condensin, whereby sister chromatids can be distinguished based on the DNA label mapping to plus or minus strands. The chemical ligation-based approach will significantly reduce barcode length compared to enzymatic ligation, which is essential for reading sufficient lengths of genomic DNA for efficient sister-chromatid-specific mapping.

With this technique, I will determine the extent to which DNA loop extrusion constricts entanglements behind condensin to promote decatenation, how much chromosome compaction by condensin affects local concentration of TOP2A and intra-chromatid catenation, and whether other potential sister chromatid resolution activities are involved.

This study will reveal how condensin and TOP2A cooperate in folding and resolving replicated chromosomes, a process that is fundamental to all life forms. The method developed in this project captures multiple targets and pairwise/multiway interaction at the single-cell level, thereby providing a powerful means to investigate the coordination of other molecular processes such as DNA replication, cohesion establishment, and DNA repair. This project will, therefore, advance technology and biological insights with implications for cell division and genome architecture.

Chemical Glycobiology to Investigate the Cell Surface Glycocode

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Glycosylation is a process in which a carbohydrate is covalently attached to a target macromolecule, usually protein and lipids. Combinatorial patterns of glycosylation that relay biological information (known as the glycocode) function in essential roles that govern human health and disease, including cancer, infectious diseases, and autoimmune disorders. Despite this, we lack detailed molecular understanding of the features that comprise the glycocode and how they contribute to biological function. Our insights about the glycocode remain limited because the non-templated complexity of glycosylation challenges our current suite of tools for studying glycoconjugates. A main limitation is our current reliance on protein-centric methodology to study cell surface glycoproteins, where combinatorial glycocode epitopes are destroyed as part of the workflow.

This “de-glycoproteomics” strategy not only limits cell surface glycoproteome characterization to a subset of glycoproteins, but also perpetuates an incomplete framework for studying cell surface glycobiology. I propose to address this critical gap in current technologies by developing a novel chemical glycobiology platform to capture the complex features of the glycocode directly from the cell surface. I will design and build a library of chemical reagents to enable selective enrichment of cell surface glycoconjugates, including both N- and O-glycoproteins; combine these custom tags with automated enrichment approaches and state-of-the-art mass spectrometry (MS)-based glycoproteomics; and interrogate the dynamic nature of the cell surface glycocode during the epithelial-mesenchymal transition (EMT) of epithelial cells with defined EMT-driven pathologies (e.g., liver, lung, kidney, pancreas, prostate, and breast cells).

My project will include three aims. First, I will utilize synthetic chemistry skills to synthesize bifunctional probes with glycan reactive handles (e.g., hydrazide and alkoxyamine groups) and phosphonate groups that can be easily enriched with well-established immobilized metal affinity chromatography methods without disrupting glycocode integrity (a major limitation of the current methodology). Second, I will establish an exciting new skillset of glycoproteomics with expertise in advanced MS instrumentation enabled by my host research group. Third, I will use my background in molecular biology to apply our platform to EMT characterization.

In all, this project will complement current efforts in my host group that focus on MS-centric strategies to improve high-throughput glycoproteome characterization. My novel approach to characterize poorly understood cell surface glycocode dynamics during cellular transformations will provide new markers of cellular states and generate new insights into how cell surface glycoproteins mediate this process. More broadly, this technology will be widely applicable to many biological questions where we and others can directly interrogate heterogeneous molecular features of the cell surface glycocode that serve as functional units of biological information.

Unraveling the Cellular and Molecular Dynamics of Adult Neurogenic Niches

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Adult neural stem cells (NSCs), a group of quiescent cells that possess the potential to generate specialized neurons and glial cells, are present in the brains of all vertebrates, including humans. NSCs are embedded in dedicated niches that are composed of heterogeneous cells, which provide a unique combination of signaling molecules and physical properties to support NSC function. Important contributions of niche cells have been identified under physiologic conditions. But how niche cells remodel to promote complex NSC activation for repair and act as cohesive unit still remains elusive.

The host lab previously identified quiescent NSC-like cells in the adult brain of genetically versatile fruit flies, which activate upon injury for regeneration, which suggests an important role for integration of multiple niche features. My proposal aims to genetically manipulate the neurogenic niche cells in vivo for the first time and characterize their coordinated functioning in the transition of NSCs from quiescence to an active proliferative state during brain tissue regeneration. My research will involve three goals. First, develop a genetic toolkit that allows me to mark NSC-interacting niche cells in homeostatic and regenerative conditions in the *Drosophila* adult brain and access molecular fingerprints. Second, manipulate identified biological and physical properties of the niche microenvironment to determine the effect on NSC activation. Third, interrogate whether changed niche properties modulate the type of cells that are regenerated using cutting-edge lineage tracing.

The group of cells surrounding NSCs, known as the niche, exhibits spatial and biological organizations that remain poorly understood. My hypothesis is that the niche functions as a unit that can be remodeled to intervene in the states of quiescence, proliferation, or fate of NSCs. To characterize and manipulate NSC niches in vivo, I will use adult NSC-like cells in the fly brain expressing the HES1-like transcription factor deadpan as a bait to mark and visualize directly interacting cells via activation of a transgenic expression system in the neighboring cells without interfering with niche cell function. This cell-cell interaction tracing system will be activated in homeostatic and injured brains to isolate niche cells and analyze their features by transcriptional and proteomic analyses. Overall, this approach will enable specific manipulation of the niche cell population for genetic experiments to elucidate the function of identified molecules.

How niche cells integrate injury signals and coordinate NSC activity in the brain is still not well understood. By genetic manipulation of the niche in vivo I aim to generate profound insight into molecular and cellular mechanisms governing niche dynamics during regeneration that will likely apply to other species and tissues and will shed light on fundamental aspects of tissue repair.

Chromatin Rejuvenation and DNA Damage Responses During Aging of Human Chromosomes

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Aging represents an inevitable endpoint in metazoans and is characterized by multisystemic functional decline. This is often linked to the accumulation of DNA damage along chromosomes with time. Elevated reactive oxygen species (ROS), in particular, are produced as a byproduct of cellular metabolism and considered among the main drivers of the pathophysiology of aging and age-related disorders. It would be expected that the process of aging and the induction of genotoxic stress would impact all levels of gene regulation, however, little is known about the differential effects that distinct stimulants of DNA damage cause in the multiple levels of genome architecture and function. Thus, an in-depth investigation of the relationship between oxidative damage and chromosome structure at the molecular level is needed.

A recent study led by the proposed host lab demonstrated that aging is characterized by abnormally high transcription elongation speeds in multiple cell types, tissues, and species, and that overexpression of histone proteins not only restores the kinetics of transcription to physiological levels, but also restricts the emergence of various aging features in human cells. The inherent link between chromatin and 3D genome structure suggests that this histone-driven 'rejuvenation' of aging cells is bound to changes in the spatial architecture of chromosomes. Still, it remains unclear how these chromatin-based interventions can counter the aging processes.

Putting these two together, I propose a study of the effects of chromatin rejuvenation on 3D chromosome architecture as well as on the genome's ability to cope with oxidative damage. To this end, I will combine ultra-resolution 3D genomics with nascent epigenome mapping in aged (senescent) and 'rejuvenated' primary human cells before and after inducing either chronic (low doses, prolonged treatment) or acute (high doses, one exposure) oxidative DNA damage. In a next step, these experiments can be complemented by in vivo dissection of rejuvenated mouse tissue at the single cell level. I expect these experiments to shed light on the molecular mechanisms and regulatory principles behind chromosomal stress responses during aging.

In conclusion, this research program will provide new insights into the crucial role of chromatin architecture during aging, while deciphering differences in the ability of aged and rejuvenated cells to respond to oxidative stress induction. Thus, possible novel approaches for preventing and managing late-life disease by epigenome regulation might emerge.

Dissecting Long-range Gene Regulation of Cell Differentiation Through Biophysical Approaches

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Proper regulation of gene expression is critical to biological function, and dysregulation is widely implicated in disease. Typical genes are controlled by transcription factors that bind both at promoter-proximal elements as well as distal enhancers as far as hundreds of kilobases away and tend to be highly cell-type specific. Improper enhancer-promoter interactions can cause devastating effects, such as when oncogenes' hijack other genes' enhancers in tumorigenesis. While enhancer-promoter associations were traditionally thought to involve stable long-range looping of chromatin to physically bridge regulatory elements, convincing alternatives have emerged from recent studies on loop-extrusion and biomolecular condensates. Therefore, the precise molecular mechanism governing enhancer-promoter interactions remains unsettled.

The roadblocks hindering our understanding are technical. First, the close spatial proximity of many enhancers to their cognate promoters cannot be resolved by typical methods (e.g., Hi-C or FISH). Second, dynamic mechanisms cannot be resolved with static methods (e.g., Hi-C or FISH). To overcome these limitations, we will combine two cutting-edge techniques developed in the host lab: region capture Micro-C (RC-MC) and Super-Resolution Live Cell Imaging (SRLCI). Whereas RC-MC enables the visualization of the local genome architecture around select target loci with unprecedentedly high resolution in space to catalog all potential enhancer-promoter pairs, SRLCI complementarily tracks defined enhancer-promoter interactions over time to chronicle dynamic reorganization. We aim to further visualize nascent transcription simultaneously by using PP7 technology. Finally, in collaboration with biophysicist Dr. Leonid Mirny (also at MIT), we will build a 3D polymer-simulation model recapitulating dynamic enhancer-promoter contact to reconcile experimental results with competing hypotheses.

These experiments will be conducted using a system producing three different lineages. By focusing on a master transcription factor for one specific lineage, we will dissect the cis-regulatory circuits promoting nascent transcription and ultimately lineage specification, which addresses a central open question in developmental biology. In terms of perturbations to this system, we will modulate the composition of loop-extruding cohesion complex and transcription proteins. My project integrates bioengineering, biophysics, computational modeling, genomics, and genetics and will answer two major questions. How do enhancers mechanistically regulate transcription? How does dynamic 3D rewiring of enhancer-promoter contacts at a key transcription factor locus drive lineage specification? Answers to these questions will fundamentally advance our understanding of physiology and disease.

Mechano-chemical Coupling in Cytoplasmic Flows: the Competition Between Self-organization and Chaos

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During embryonic development, the cytoplasm separates into distinct compartments prior to cell division. This partitioning occurs spontaneously and in the absence of external stimuli or physical boundaries (i.e., the plasma membrane). Regulated by microtubules, the partitioning forces can lead to large-scale cytoplasmic flows. These flows, in turn, redistribute cytoplasm and nuclei, which define the new cell centers. Meanwhile, the constituents of the cytoplasm are free to move. Yet, they must relocate to a suitable position before the subsequent cell division. Reliant on the capacity of the cytoplasm for self-organization, the coordination of this redistribution results from the interplay between chemical and physical processes. While spatially synchronized chemical oscillators determine the timing of the cell cycle, active processes facilitate flow patterns that robustly redistribute the constituents within the cytoplasm. The potential for chaos has been hypothesized to pose an inherent restriction to development, which relies on maintaining order in the chemical oscillations as well as cytoplasmic flows. While simulations of the cell cycle oscillator confirm its potential for chaos, experiments have yet to confirm this possibility. Additionally, the consequences of a perturbed cell cycle oscillator on the spatially reorganization of the cytoplasm are unknown.

Recent studies showed that active fluids can transition to turbulent flows even in the absence of inertial forces. Unpublished results in the host lab confirmed this for flows in reconstituted cytoplasm of Xenopus laevis. I aim to investigate the role of both cellularization and coupling of active flows with the cell cycle oscillator as strategies to avoid chaos. I hypothesize that the mechano-chemical coupling between the cytoplasm and the cell cycle actively regulates the transition to chaos.

First, using microfluidics, I will generate droplets containing cycling cytoplasmic extract. By varying droplet size and boundary properties (e.g., stiffness), I will explore the effects of boundary interactions on internal flows. Second, I will extend my investigation to a multiple droplet system to explore the effects of direct or indirect coupling (e.g., chemical or mechanical) of adjacent droplets and their effect on internal flows. Third, I will perturb timing and spatial coupling with the cell cycle in the first two stages. I will evaluate the robustness of these systems by analyzing their response to external perturbations (e.g., mechanical deformations or localized temperature increases).

In summary, this project will investigate the competition between the mechanisms of self-organization and the antagonizing transition to chaos in cytoplasmic flows. This research has the potential to explain why some embryos develop as syncytial masses while others divide into individual cells.

Understanding the Role of Endometrial Compartmentalization in Menstruation

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The lining of the uterus (endometrium) is the most powerful regenerative system in the human body. It undergoes monthly shedding during menstruation and subsequent regeneration, and completes up to 400 cycles over the human reproductive lifespan. Studying menstruation is not only critical for hundreds of millions of people affected by its associated disorders, but it can also provide crucial insights into human tissue regeneration. However, the molecular mechanisms of endometrial shedding and regeneration remain poorly understood.

The endometrium is compartmentalized into two layers: the functionalis, which sheds during menstruation, and the basalis, which remains and proliferates to regenerate the functionalis. This compartmentalization is essential for physiological endometrial regeneration, as damage to the basalis is associated with non-regenerative outcomes (fibrosis). However, we currently lack a detailed molecular definition of functionalis and basalis that explains their functional differences.

I propose a dynamic and multimodal characterization of endometrial compartments throughout the menstrual cycle. My studies will leverage the African spiny mouse (*Acomys cahirinus*), a rodent that was recently discovered to menstruate[5], which provides a unique platform for experimental dissection of menstruation. My research project has three aims. First, I will use spatial transcriptomics to identify gene expression signatures that distinguish the functionalis and basalis within the cycling endometrium. In parallel, I will assess two potential functional differences between the functionalis and basalis (Aims 2 and 3). In Aim 2, I will test a longstanding, but largely untested hypothesis in the field: that secretion of matrix metalloproteinases (MMPs) within the functionalis initiate endometrial shedding by degrading the extracellular matrix holding the tissue together. I will assess MMP protein expression and activity profiles across endometrial strata and evaluate whether MMP activity is essential for menstrual shedding using specific MMP inhibitors. In Aim 3, I will test whether differing membrane potentials between functionalis and basalis cells contribute to their distinct behaviors during menstruation.

Cell membrane potentials are emerging as important determinants of cellular behavior, and endometrial cell membrane potentials are known to fluctuate throughout the menstrual cycle. I will assess membrane potentials in the endometrial strata over the menstrual cycle by intravital imaging and perturb ion fluxes with specific ion channel inhibitors to assess their functional contributions to endometrial cell behaviors. Taken together, this multimodal characterization of the cycling endometrium will identify the unique properties of its layers and provide novel perspectives for the holistic understanding of its regenerative processes. These findings will hold tremendous potential for future applications in regenerative medicine and reproductive health.

A Nucleus-driven Paradigm of Mechanotransduction and Morphogenesis

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Nucleus states such as shape and stiffness regulate gene expression, and the nucleus has emerged as a key mechanotransduction organelle. However, we lack an understanding of the role of nucleus states and mechanotransduction during tissue development in vivo. Previous studies on mechanotransduction have established that the nucleus senses mechanical forces applied to the cell to modulate gene expression. Doing so, these former studies have assumed that such response is 'passive' as they did not fully consider the initial state of the nucleus prior to force transmission.

However, based on accumulating works and my own research, the nucleus is a dynamic organelle that constantly moves, deforms, and changes its mechanical properties or subnuclear organization. As nuclear force transmission and gene expression depends on nucleus states, I hypothesize that the initial states of the nucleus (e.g., shape, position, stiffness) and subnuclear organization play a key role in modulating nuclear mechanotransduction and consequently any processes linked to mechano-transduction, such as tissue morphogenesis. To address this outstanding hypothesis, I chose the Drosophila epithelial tissue of the pupal dorsal thorax as a model. This tissue is composed of more than 10,000 cells, whose various dynamics regulated by gene expression and mechanical forces have been characterized. In addition, it is amenable to physical manipulation, spatialomics, and optogenetics.

Building on these resources, I propose the following research plan. I will determine nucleus states at tissue scale during development by lattice light-sheet microscopy. I will then alter nucleus states (position, movement, shape, size) within the tissue at defined time points by optogenetic loss/gain of function of known regulators of nucleus states. Then, I will compare the spatial transcriptome of tissues with/without alteration of nucleus states to determine whether the expression of the genes involved in transcription or mechanotransduction is changed. Then, the mechanisms controlling such transcriptional changes will be studied by determining gene loci localization and epigenetic modifications. Next, I will apply mechanical forces to the tissue with/without alteration of nucleus states, using physical and optogenetic tools. Then I will compare the spatial transcriptome to determine whether the initial nucleus states modulate mechanotransduction. Last, I will assess cell and tissue morphogenesis with altered nucleus states to uncover the impact of nucleus states on tissue morphogenesis.

The Bellaïche lab has developed state-of-the-art techniques required for this research. I expect the combination of my expertise (nuclear dynamics, live cell imaging), the model system, and the host lab's know-how will synergize to define novel concepts that broaden the horizons of mechanotransduction and morphogenesis.

Non-contact Measurements of Electrochemical Potentials in Skin

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The ability to control electronic charge is remarkably useful, as it underpins many consequential technologies. In humans, for example, electrical measurements have long been proposed to differentiate human physiology; but, these measurements are often confounded. When a current passes through an electrode-skin contact, there are significant variables: skin moisture, contact impedance (understood as the unpredictable accumulation of ions), the choice of contact medium, and pressure/contact variability. This is common among all known biological/electrode junctions. This fact – the effect of unknown impedance at the biological junction – has left an unexplored gap in all electrophysiology. Although many researchers are familiar with ECG (heart), EIS (ion motion) or EEG (brain) measurements, these rely on the subtraction of high-frequency signals, which are not strongly damped by the impedance of the body. Yet, this technique ignores nearly all near-DC electrical measurements, which remain unexplored.

This is surprising, as they are assumed to be pivotal in wound healing in mammals and essential in controlling the growth of worms. I propose to measure the voltages with no need to contact the biological interface by removing the electrode, and instead, developing a capacitor arrangement, a Kelvin probe. In doing so, we remove the problems associated with measuring the near DC regime. I will design bio-Kelvin probes that measure biological interfaces through varying capacitance, rather than ohmic contacts. By vibrating the electrode above the area of interest, and thus, varying the capacitance of the system, it is possible to determine the potential of skin without touching it. I will translate the theory of this measurement from condensed matter physics to human skin and develop a new relevant theoretical framework. We will use both human subjects and animal models in the verification of our understanding.

I will open a new paradigm in electrophysiology by investigating near DC potentials in human skin. I will map this potential landscape using non-contact array electrodes using real-time and long-term measurements. These measurements will be correlated to the maintenance of the transepithelial potential gradient with specific applications in understanding wound healing and the verification of nerve density.

This research will advance our understanding of electrophysiology in humans in the near DC regime. Our findings will be key to developing technological developments for next-generation bioelectronic devices, and will open a new range of frequencies to explore. I believe my experience in solid-state physics and moving to a lab with experts in human electrophysiology will open a new window to the sophisticated and complex mechanisms of charge transport in living organisms, particularly in human skin.

Exploring Novel Riboswitches in Plants: Uncovering New Regulatory Mechanisms for Plant Metabolism

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Riboswitches are regulatory RNAs with specific structures that detect metabolites/ions to regulate RNA processes rapidly and precisely, and they serve as regulatory modules in multiple metabolic pathways of bacteria. However, identifying riboswitches in plants is challenging due to limitations in identification methods and incomplete UTR annotation. Tomato is an ideal model for studying plant metabolism due to the presence of major metabolite classes, and my previous research has demonstrated that RNA structure plays a critical role in tomato.

We will explore novel riboswitches in tomato and identify previously unknown plant metabolite riboswitches. This research will have a broad range of routes to impact noting the role of metabolic pathways in determining nutritional, resilience, and pharmacological qualities in plants. The research will involve three aims. First, we will aim to generate in vivo RNA structurome landscapes for different developmental stages in tomato. We will do so using in vivo RNA structure profiling methods developed in my host lab and further determine RNA structure alterations across developmental stages. Second, using deep-mining novel riboswitches in transcriptome-wide structural comparisons I will develop cutting-edge predictive models based on deep learning to systematically explore non-trivial relationships between RNA structural alterations and RNA processing changes. Third, validating and optimizing riboswitches identified from tomato, we will mutate RNA structures and validate identified riboswitches.

By working with biophysicists in Prof. Zoe Waller's group, a long-term collaborator of my host lab, we will assess the binding of identified riboswitches with metabolites. We will optimize the RNA structure to achieve either metabolite hypo/hyper-sensitive plants. We hypothesize that riboswitches play a crucial role in regulating animal and bacterial metabolism and that they are also globally present in plants. Finally, traditional metabolic regulation is challenged by the redundancy of functional genes, and overexpression may lead to gene silencing due to feedback. We will identify specific riboswitches, thus, paving the way for new regulatory mechanisms to control plant metabolites.

Novel AI-based methods will allow us to identify riboswitches with high efficiency and accuracy at the high-throughput manner. We will use in vivo RNA structure profiling in tomato using chemical structure profiling methods, and then employ deep mining novel riboswitches in plants via AI approaches. We will aim for validation of riboswitches with plant transgenics, biophysics, and metabolism.

03 Physiology

Investigating the Effects of Chronic Stress on Gut-brain Interactions

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Stress can lead to, or exacerbate, several dysfunctions within the gastrointestinal tract that may manifest as alterations in motility, permeability, secretion, as well as visceral hypersensitivity and hyperalgesia. First level response to stress activates the autonomous nervous system releasing catecholamines, such as adrenaline and noradrenaline. If the stressor persists, the hypothalamic-pituitary-adrenal axis is engaged and cortisol is released from the adrenal glands. Actions of the opposing parasympathetic system are mainly mediated via the vagus nerve. Its afferent fibers, relay sensory information from the periphery to the brain, while vagal efferents carry instructions from the brain to the gut. Vagal efferents, inhibited by stress, are a major component of parasympathetic activity and act to decrease intestinal inflammation and strengthen intestinal barrier function, as shown in animal and human studies. In mice, mild chronic stress increases hypersensitivity in sensory gastric vagal afferents. Although the inhibitory effect of stress on vagal activity is known, the exact mechanisms of dysfunction remain elusive.

Since vagal neurons are heterogenous, I aim to decipher which cell-types are implicated in the dysfunction that occurs after long-term stress exposure, whether cell-types can be attributed to a particular feature of dysfunction, such as hypersensitivity and motility, and how these alterations are reflected in the brain leading to efferent activity modulation. I plan to utilize refined experimental tools developed in the group of Prof. Anikeeva to remotely induce magnetothermal stimulation of corticosterone-release in mice chronically and non-invasively.

Macro and micro-scale criteria of gut dysfunction will be monitored via assessment of structural, barrier integrity and motility changes. To identify implicated afferent subtypes that exhibit altered activity after chronic stress exposure, I will perform single cell sequencing of the nodose ganglion (where cell bodies of vagal afferents converge) and compare the immediate early gene expression patterns to that of the control mice. Based on the identified altered activity patterns, I subsequently investigate whether cell-type targeted optogenetic modulation of the identified affected vagal afferent subtypes are necessary and/or sufficient for inducing or inhibiting parameters of dysfunction. Additionally, to identify how stress exposure is reflected in the brain, I will look for neuronal activation markers using whole-brain clearing and imaging with a particular focus on projection targets of vagal neurons.

I aim to understand which part(s) of the gut-brain circuitry are affected by chronic stress, where the information relay to the brain is disrupted and to identify the neuronal and hormonal junctions that mediate gut hypersensitivity and altered function.

Analysis of Molecular Changes of the Nervous System in *C. elegans* Upon Circadian Rhythm Entrainment

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Caenorhabditis elegans are ectothermic and thus, are subject to diurnal and seasonal changes in temperature, specifically of soils, where they naturally reside. *C. elegans* sense temperature changes through specific thermosensory neurons and respond through changes in intracellular signaling. These neurons are essential for the regulation of longevity (Lee & Kenyon, 2009). In the laboratory setting, single and multiple, short-term heat shocks have been shown to increase lifespan in *C. elegans* (Zhou et al, 2011). The phosphoproteome of adult *C. elegans* after short-term mild heat stress showed a wide range of signaling changes, notably to proteins associated with lifespan determination (Huang et al, 2020). *C. elegans* can be entrained to a circadian rhythm by the application of temperature cycles (Olmedo et al, 2012). However, it remains unclear if temperature-induced circadian rhythms have a protective effect on longevity compared to a single short-term heat shock. Thus, we aim to elucidate whether circadian rhythms entrained by temperature cycling have a positive effect on longevity through the analysis of the changes to mRNA expression and the proteome, specifically in the nervous system.

Our hypothesis is, circadian rhythm temperature shifts increase *C. elegans* longevity and result in mRNA and proteome changes to the nervous system. Our research will pursue two main questions. First, how do oscillating circadian-like temperature shifts affect *C. elegans* longevity? To address this, we will use an automated platform with precise temperature control. We will employ circadian rhythm paradigms that mimics naturally occurring temperature oscillation from to 12°C to 22 °C and compare those to a constant state as well as a single heat shock to determine their impact on *C. elegans*' longevity. Longevity will be assessed by measuring locomotion, touch response, and pharyngeal pumping. We will determine the circadian entrainment paradigm that has the most beneficial effect on longevity. Second, which pathways are activated/repressed in the nervous system during and after circadian temperature entrainment? We will utilize Ribotag technology with RNAseq and TurboID fused to a fluorescent protein with LC/MS to identify actively translating mRNAs and proteins and post-translational modifications, respectively in the nervous system using neuron-specific promoters. Inducible TurboID in *C. elegans* is established (Artan et al, 2021). We will determine the overlap between the RNA and proteome datasets and perform pathway analyses using computational tools. Promising candidates will be validated by tissue-specific RNAi, CRISPR-Cas9 technology and functional assays to elucidate underlying mechanisms. Our goal is to elucidate neuronal molecular mechanisms regulating the responses to naturally occurring temperature oscillations and their effect on the animal's lifespan.

04 Life and Evolution

Unraveling the Genetic Basis of Natural Variation in Secretion Pathways

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Protein secretion is a universal process in cells. Apart from its vital role in cellular physiology, protein secretion is also at the basis of several important diseases as well as industrial applications in which cells are used to produce proteins of interest. Despite its relevance, it is not known whether there are genetic and biochemical differences among individuals in their capacity to secrete proteins, and if so, what the genetic underpinnings are.

In this project, I will address the current knowledge gap by exploring *Saccharomyces cerevisiae* (Brewer's yeast) biodiversity to identify natural alleles that underlie protein production and secretion variation. My hypothesis is that protein secretion is a variable, polygenic trait that evolved in parallel across different genetic lineages. Because of the numerous evolutionary conserved signaling pathways, its ability to introduce post-translational modifications, and the genetic toolbox available, *S. cerevisiae* is a fantastic model organism for eukaryotic physiology and genetics, and the ideal candidate for the study of protein production and secretion mechanisms. Notably, *S. cerevisiae* is also a phenotypically diverse species, exhibiting several niche adaptations that can be traced to the genomic level. Surprisingly, preliminary results from the host lab indicate that there is a high diversity (i.e., 3-5 fold) in protein production and secretion among natural *S. cerevisiae* strains.

Using the genetic toolkit in yeast, I will unravel, for the first time, the genetic and biochemical basis for differences in protein production and secretion, and identify the bottlenecks in protein secretion. I will achieve this through 3 specific aims. First, I will investigate the natural biodiversity for protein secretion, by using the host lab's unique yeast collection, and vast experience with large-scale screening and investigation of complex traits. Then, I will use a 'Round Robin' QTL mapping approach on ten genetically diverse strains to identify novel alleles affecting protein secretion and homeostasis to expand our understanding about this intriguing and complex trait. Finally, I will apply this knowledge to develop a working model of eukaryotic protein secretion and homeostasis and develop two robust strains for increased protein secretion.

The newly acquired knowledge will not only pave the way for elucidating protein secretion patterns in related human conditions and the virulence phenotype of other microorganisms, but might also identify new drug targets and strategies for improved secretion by microbial cell factories.

Adhesive Mechanism of Poison Frog Tadpoles: How to Attach on Soft and Wet Surfaces

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Adhesion is critical for survival in many animals: ants have to climb vertically or upside down on plants in search of food, and barnacles need to withstand strong waves during low tide. Animals have evolved a myriad of attachment devices and have inspired novel biomimetic adhesives. Most studies to date have measured the performance of animal attachment organs on rigid and dry glass and polymer substrates. However, a major difficulty for medical adhesives is to adhere to soft, wet tissue or skin and avoid damage when removed.

Therefore, understanding how animals achieve controllable adhesion to soft and wet surfaces is essential for the development of biomimetic tissue and skin adhesives. The attachment of poison frog tadpoles on the soft and wet skin of their parents represents a novel and fascinating model system. Upon hatching, tadpoles attach to the back of their parents, who carry them from the clutch near the ground to the arboreal aquatic nursery. Poison frog species differ greatly in the relative size of frogs and tadpoles, number of tadpoles carried, and height of the nursery sites. Currently, the only proposed attachment mechanism is viscous adhesion via mucus surrounding the frogs and tadpoles, but this is likely insufficient due to the nature of viscous adhesion.

I hypothesize that suction, in combination with mucus, and other strategies (e.g., interlocking of soft structures) are involved and may vary with the ecology and behavior of each species. This interdisciplinary project will reveal the attachment system in poison frog tadpoles by integrating biophysics, functional morphology, and comparative evolutionary biology. First, I will analyze the kinematics of frog jumps from high-speed videos and quantify the adhesive force required for the tadpole to remain attached. I will then compare this force with the maximum adhesive force directly measured by pulling the tadpoles off frogs in vivo. Second, I will identify which ventral regions the tadpoles use for attachment on smooth and micro-structured surfaces using interference reflection microscopy. Third, I will examine the morphology and microtopography of relevant frog and tadpole structures at the observed contact zones (e.g., mouth and skin surface sculpturing) using advanced microscopy (micro-CT, SEM, confocal).

Next, the observed variation in morphology will be translated into replicas and 3D-printed models for adhesion tests in combination with mucus on artificial materials. I will also measure the properties of the mucus using rheology and micro-rheology. Finally, to identify evolutionary drivers of the adhesion-associated adaptations, I will test how tadpole size, numbers, and nursery height determine the morphology of attachment systems in different species using a phylogenetic comparative analysis. The project will not only provide insights into a novel, wet attachment system, but also potential inspiration for medical adhesives.

Early Earth Conditions as a Selector for Functional RNAs

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Prebiotic scenarios rely on the emergence of sustained catalysis. One candidate molecule is RNA where various oligomerization mechanisms and numerous catalytic RNAs (ribozymes) have been discovered. However, a spontaneous transition in between seems improbable due to the scarcity of functional sequences among random strands. For instance, the medium-sized class I ligase is estimated to occur once in 1018 sequences. This leaves little opportunity for a ribozyme to appear by chance, lead to the formation of another ribozyme, and kick-start molecular evolution. The major transition, thus, begs for some form of external constraints that enrich functional sequences among the random ones.

I suggest that geomaterial-RNA interactions could have acted as a filtering mechanism. Such interactions have been shown to catalyze RNA oligomerization, reduce RNA hydrolysis and select for longer oligomers. However, in the transition from random to functional RNAs, the geological context has so far been neglected. Since all catalytic RNAs and their substrates are partially folded, we expect their adsorption on geological surfaces to differ from that of unfolded or completely double-stranded sequences.

My research plan consists of three parts: first, parsing of the folding space and proof of principle of the fold-dependent adsorption; second, inhibition and recovery of ribozyme activity within random pools; third, emergence and survival of reaction networks in random RNA pools. In part one, I will design a repertoire of folds from statistics of known ribozymes and randomly picked motifs. I will quantify the fold-dependent adsorption on different surfaces (rocks, minerals, and clays) and screen the effects of environmental conditions, such as temperature, pH, and salt during the adsorption and washing steps. Secondary structure predictions and thermodynamic adsorption models will serve to fit and predict adsorption of RNA folds. In part two, I will study how binding of random oligomers to ribozymes leads to inhibition by probing the activity of various ribozymes within mixtures of increased randomness. The degree of enrichment of folded sequences required for function and the results from part one will enable me to establish how selective adsorption on geomaterials can restore activity. In part three, I will explore conditions to sustain autocatalytic reaction networks in an open reactor that mimics connected rock cracks. Known reaction networks mixed with random pools will be submitted to rounds of adsorption on different minerals and washing. Using the findings of parts one and two, I will determine the geological environments that enabled the emergence of sustained autocatalysis despite inhibition and side-reactions caused by random RNAs. This project will provide a quantitative basis to assess the spontaneous emergence of function from random RNA pools and will identify the most plausible geological conditions for this crucial transition during the origin of life.

Following Dance Vibrations on a Swarm: the Emergent Mechanics of Honey Bee Collectives

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When animals form groups they display emergent properties that can be quite different from those shown by individuals. For example, fire ants self-assemble into dynamic structures to face adverse conditions. This study looks at how a structure composed entirely of interlinked and interacting honeybees can transmit vibrations as part of the waggle dance communication system. When honeybees move nests, they form large, organized comb-less clusters called swarms. Swarms consist of several layers of bees, and the structural integrity of the swarm is maintained by bees rearranging themselves. Since combs are needed for reproduction, bees need to quickly transition out of the swarm stage by choosing a new nest site. This decision making is mediated by waggle dances. A dancer bee attracts followers to itself and communicates information to them via a waggle dance. Substrate vibrations are an important part of dance communication, and previous work has assumed that combs are required to carry these vibrations. Data from one honeybee species (*A. dorsata*), however, strongly hints that even swarming bees communicate using waggle dance vibrations, which must therefore transmit through their interlinked bodies.

I propose to study the vibrational characteristics of the dance signal on these two different substrates and also on the receiver's vibration detecting sensory system. To study substrate-dependent transmission properties, I will measure the dance vibrations on combs and swarms using a combination of laser Doppler vibrometry and behavioral observations. Next, I will use electrophysiological measurements to test the tuning of the leg mechanosensory organs for dance vibration detection. To test that the cue relevant to dance detection is indeed substrate vibration, I will disrupt the leg mechanosensory organ and test whether dances can still be detected on either substrate. Lastly, I will use mathematical modeling to understand the physical principles governing vibration transmission on combs and swarms.

Biophysical and mechanical explorations of bee swarms have usually focused on the principles that enable individual bees to form these large collectives. This study will be the first to explore honeybee swarms as a material that transmits vibrations and will provide a new direction to explore the honeybee swarm as an active material. The swarm can be considered as a metamaterial, whose mechanical properties can differ based on the arrangement and interaction of individual bees. Importantly, we will test how interconnected bees can provide a flexible, yet reliable medium for communication and decision making. Thus, honeybee swarms will form a template that can be extended to design autonomous systems that can self-organize and communicate. These findings will have implications in fields ranging from animal communication to swarm dynamics and robotics.

Evolutionary Dynamics and Self-organization in Active Populations

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Evolutionary ecology has recently benefited from fruitful collaborations between theoretical statistical physicists and microbiologists. Repeatable experiments on biofilms are a test bench for our theoretical understanding of the spatial structure of evolution. For instance, it was shown that when cell division drives the growth of dense cell colonies, spatial constraints substantially shape evolution, leading to spatial genetic segregation. Most organisms, however, do not spread out because of cell division but instead follow complex nonequilibrium dynamics characterized by active motility. These dynamics lead to complex forms of organization, which have been extensively studied (in the absence of evolutionary forces) within the new field of physics called active matter. A major yet unexplored question is how motility impacts the genetics of growing populations. How does spatial evolution occur in the presence of motility? What is the fate of genetic segregation in actively spreading populations? To address these questions, the physics of active matter has to be coupled with the genetics of populations, in order to study the interplay between active motility, genetic mutations, and natural selection. To this aim, I will combine microscopic models of active particles with branching processes representing cell division. Using stochastic calculus, field-theoretical methods, and numerical simulations, I will study the emergence of spatial patterns and genetic sectoring in such models. Secondly, I will account for different cell types and ecological interactions, modeled via reaction-like terms in the spirit of generalized Lotka-Volterra models. The goal of this second part will be to study how such asymmetric reaction terms impact both the motility-induced pattern formation and the genetic sectoring.

The project will be hosted in the group of Julien Tailleur, who is a world expert on active matter. He has discovered the motility-induced phase separation. Recently, he has shown how this phase transition can promote self-organization in multi-strain bacterial colonies. The project will also benefit from the expertise of Jeff Gore at MIT, an expert in experimental microbial ecology, and David Nelson at Harvard University, who discovered how fluctuations can lead to genetic sectoring in expanding colonies. Connections with experimental systems will be evaluated whenever possible. The expected outcome is a predictive theory of spatial evolution in active populations, such as motile dividing cells. The challenge is to enrich the conceptual framework of conventional active matter to account for evolutionary forces and genetic correlations. A potentially strong impact is foreseen for different biological fields, from developmental biology to ecology.

05 Neuroscience

Brain-wide Mapping of Neurochemical Activity Using Liposomal Nanoparticle Reporters

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Multiple neurochemical species that play functionally distinct roles in neural circuitry interact to influence the large-scale activity of the brain. Understanding the spatial and temporal characteristics of such chemical signaling, thus, becomes crucial for building mechanistic models of brain function. The technology for measuring neurochemical activity in vivo is still incipient. Fluorescent probes allow several neurotransmitters to be imaged in optically accessible structures, but mapping the topography of neural signaling events in deep tissue is not possible with optical methods, or with older point measurement techniques.

Over the past few years, Alan Jasanoff's lab has led efforts to create probes for mapping neurochemical events using molecular-level functional magnetic resonance imaging ("molecular fMRI"). The scope of such experiments has, however, been limited by the modest sensitivity provided by the existing probes, which must be applied at concentrations that substantially exceed physiological neurotransmitter levels.

My project aims to provide a platform technology for noninvasive neurochemical imaging with improved sensitivity that will initially target monoamine transmitters. Our idea is to develop a fundamentally distinct and more sensitive architecture for neurochemical imaging in the nervous system. The new probes are liposomal nanoparticle reporters (LisNRs) that give rise to substantial stimulus-dependent MRI contrast changes even at nanomolar concentrations. To achieve sensitivity to neurotransmitter targets, we will establish ligand-responsive LisNRs that employ peptide-based pores in the liposome membranes. Neurotransmitter-dependent contrast changes occur when protein domains block pore permeability in the absence of free neurotransmitters, but not in their presence.

In our research, we will optimize this mechanism for the detection of behaviorally relevant interstitial dopamine and serotonin concentrations. Next, we will optimize the distribution profile of liposome-based dopamine and serotonin LisNRs when introduced into the brain using minimally invasive procedures. Then we will validate these probes by molecular fMRI in live rat brains. By creating sensors for neurochemical targets, optimizing their delivery to brain tissue, and performing functional imaging studies in rats, we will define stimulus-induced signaling profiles that are inaccessible to other experimental means and establish a platform that can be adapted for imaging additional targets in optically inaccessible tissue. Our experiments will set the stage for the broad application of LisNR probes for qualitatively novel characterization of neurochemical dynamics relevant to behavior and neural circuit function at a brain-wide scale in many species, including potentially humans.

The Biophysics of Causality – Sensing and Control of Network Dynamics by Single Neurons

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Each neuron in the central nervous system establishes thousands of synaptic input and output connections, which form networks that generate the brain-wide activity patterns that underlie behavior. However, a fundamental gap remains in our understanding of how neurons, through local plasticity mechanisms, can organize themselves into behaviorally useful circuits. How can a single cell discern its own contribution to these global activity patterns? One radical idea is that neurons form control loops with their networks, using the feedback received following their own action potential firing to gauge their causal influence on network dynamics, and then adapting to this feedback in order to drive the network to a specific function.

This project aims to bridge the gap between cellular physiology and network computations by examining the precise role that an individual neuron's output plays in the control of its inputs, and the interplay of such control with the neuron's own plasticity mechanisms. I will leverage recent advances in optical imaging and molecular perturbation techniques to study how single hippocampal CA1 pyramidal cells influence and are influenced by their local network during place field formation, a well-characterized and behaviorally relevant form of plasticity. I will use state-of-the-art two-photon 3D acousto-optic deflector microscopy to longitudinally monitor the synaptic inputs and outputs of a single CA1 neuron by co-expressing recently developed high-affinity presynaptic glutamate sensors with red-shifted calcium indicators. After characterizing a neuron's nominal input and output dynamics during plasticity, I will disrupt glutamate release originating from this single neuron by using precise molecular tools, such as inhibition of presynaptic vesicle fusion or single cell conditional deletion of VGLUT1, sparing all other presynaptic processes.

The driving hypothesis is that individual neurons sense their role in network computations by actively regulating their own input through action potential firing. A major objective will therefore be to characterize the effects of output blockade on key physiological properties of CA1 hippocampal neurons, such as dendritic amplification and synaptic stability. In addition, by simultaneously recording the activity of surrounding neurons, I will be able to quantify the role of the single manipulated neuron in the local network, providing new evidence for several previously hypothesized mechanisms, such as lateral feedback inhibition in the regulation of hippocampal memory formation. Unraveling the control loops that neurons form with their networks will offer unprecedented insights into the interplay between a neuron's plasticity rules and their role in shaping network function. By defining a novel mechanism for neuronal sensing of their causal network contributions, this project will lead to fertile ground for future development of biological learning algorithms.

Ultrasound Neuromodulation: Search for the Physical Origin

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Ultrasound (US) is an exciting tool for biomedical purposes due to its ability to non-invasively interact with matter deep inside our body with relatively high precision. These interactions can be exploited at low intensities for imaging, or at high intensities for destruction of tissue. The intermediate US intensity range, however, presents a grand challenge, since it affects biological cells through a complex interplay of several forces, including: the acoustic radiation force (ARF), acoustic interaction force (AIF), and forces from acoustic streaming (AS) and cavitation. These US-induced forces can lead to significant biological responses enabling, for example, neuromodulation, biomarker release, sonoporation, and angiogenesis. However, despite their enormous potential, these forces remain poorly understood due to the entanglement of the underlying mechanisms, as well as the fast timescale and small length scales that make experimental analysis challenging.

Neuromodulation with US (USNM) has been demonstrated in vitro and in vivo and is particularly interesting, as it could yield significant breakthroughs in neuroscience and neurological treatments. Potential uses include suppression of epileptic seizures or symptoms of psychiatric disorders. I plan to first discover which acoustic forcing mechanisms are responsible for USNM, and to what extent, and then determine the magnitude and nature of the involved forces. Currently, several mechanisms are hypothesized to be responsible for USNM, including: pressure and velocity variations on the microsecond timescale, ARF, AIF, and AS. In a typical US, these mechanisms act concurrently. However, standing-wave US fields, combined with varying the position of neurons, media properties, and US parameters, could enable a decoupling of the mechanisms.

The experiments developed in the project will be based on standing-wave lab-on-a-chip acoustofluidics and will provide well-defined acoustic environments for neurons to interact with each promoting a different isolated mechanism of acoustic forcing, and thus, revealing its role in USNM. The forces on neurons at the cellular level will be investigated through particle image velocimetry, traction force microscopy, and fluorescence resonance energy transfer force sensors. Computational models of a neuron in US for quantification of forces will be developed and calibrated through extensive material characterization of neurons and the surrounding media. The project aims at discovering the main underlying mechanisms of USNM and could therefore provide the missing foundation, which would have an enormous impact on the development of USNM and its translation to clinics and neuroscience research. Furthermore, the developed methodology could pave the way towards understanding how US interacts with biological cells in the context of other, equally promising biological responses, such as angiogenesis or release of cancer biomarkers.

Memory Trace in a Neuron: Dissecting Dendritic Mechanisms of Learning

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Dendrites, the elaborate tree-like structure of neurons, are the key sites of neuronal computations by processing synaptic inputs and transmitting information to the cell body of neurons. Their significance is underscored by their observed degeneration and dysfunction in numerous neurodegenerative diseases. While interdependent somatic and dendritic activity has been suggested to implement functions that determine the input-output relationships in individual neurons, it is still technically impossible to capture dendritic computations in isolation from the influence of somatic activity in the intact brain. Therefore, we still do not know whether or how soma and dendrites operate independently or collaboratively to achieve cognitive functions in behaving animals.

To address this question, it is necessary to monitor and manipulate the activity of soma separately from dendrites in behaving animals. I will combine in vivo dendritic voltage imaging, intracellular recordings, and manipulation of somatic membrane potentials in hippocampal pyramidal neurons to elucidate the potential roles of dendritic computations in neuronal plasticity underlying learning and memory. My research will leverage the formation and subsequent stabilization of spatial representations in hippocampal CA1 pyramidal neurons as a model of neuronal plasticity associated with spatial learning. The plasticity rule behind this process, known as behavioral timescale plasticity (BTSP), is characterized by dendritic plateau potentials and somatic burst spiking and is believed to involve somatic and dendritic plasticity, the precise roles of which remain enigmatic in vivo.

I will first reveal the relationships between dendritic computations, somatic spikes, and dendritic plateau potentials throughout spontaneous place field formation and subsequent refinement of the spatial information associated with sharp-wave ripples (SWRs), the high-frequency oscillations in the hippocampal local field potentials (LFP). To achieve this, I will conduct two-photon voltage imaging of dendritic activity with ultrafast acousto-optical microscopy and simultaneous whole-cell patch-clamp recordings from their soma together with hippocampal LFP recordings while mice explore a novel environment. Next, I will manipulate somatic membrane potential to prevent soma from emitting spikes and study dendritic activity. This revolutionary approach will clarify for the first time whether and how somatic spikes and plateau potentials are causally related to dendritic computation and propose a potential role of dendrites in spatial memory formation. The innovative combination of technologies that I am pioneering will open up a whole new world in understanding soma-dendrite computation in general and may provide novel insights into the pathologies caused by its abnormalities.

Does the Striatum Use Cerebellar Motor State Predictions to Guide Rapid and Complex Movement?

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The brain's ability to control fast, yet complex and tightly coordinated movements, like guitar solos, is remarkable. Any sensory feedback ostensibly guiding such rapid movements is necessarily delayed, as it takes time for nerve impulses to travel to and fro, between brain and fingertips. This begs the question: How does the brain mitigate sensory delays (and noise) when generating rapid, yet complex movements? One possibility is that such movements are guided by near instant, "0-lag" estimates of the current state of the body, rather than by slow "actual" sensory feedback. A brain region commonly believed to compute model-based motor state estimates is the cerebellum.

I hypothesize that fast, yet complex movement sequences generated by the dorsolateral "sensorimotor" striatum (DLS) are guided by 0-lag body state estimates computed by the cerebellum (CB) and relayed to DLS via the intralaminar thalamic nuclei (ILN). To verify this hypothesis, I will attempt to demonstrate that the signal transmitted via the CB-ILN-DLS pathway is congruent with model-based body state predictions. Second, I will aim to confirm that the pathway is critical to coordinate and control fast, complex, and DLS-dependent movement sequences with precision. To achieve these aims, I propose to record (using electrophysiology and optotagging) the activity of DLS-projecting ILN neurons, as well as to reversibly inactivate (using chemogenetics) the CB input to ILN-DLS "relay neurons," in freely moving rats executing fast, complex, and confirmed DLS-dependent movements.

If the hypothesis is true, the activity of the ILN-DLS neurons should accurately reflect current body state in detail and with little to no temporal lag until unpredictable deviations are introduced to the sensorimotor state, or the neurons' CB input is blocked. Once the CB input to ILN is blocked, rats should further misjudge the position of their limbs in space, and mistime, over-shoot or undershoot, their movements in particular when they are moving fast.

CB and DLS are critically implicated in motor control in health and disease, but are thought to principally interact through the cerebral cortex. The cortex-DLS loop has been linked to efficient model-free action selection driven by state-dependent action policies, whereas the cortex-CB loop is believed to facilitate flexible model-based online control of ongoing movements. This study aims to reveal an as yet unexplored mechanism, by which model-based CB predictions may be integrated into model-free DLS policies without cortical involvement. The exploration of such subcortical CB-DLS interactions is potentially important and may pave the way toward discovering novel causes, mechanisms, and treatment options with regard to the many movement disorders associated with CB and DLS dysfunction.

Reward-based Learning of Assemblies in Olfaction

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It is widely accepted that groups of pyramidal neurons (PNs) that display synchronous activity and strong recurrent synaptic connectivity, often referred to as an 'assembly,' represent a specific stimulus or concept, and thus, act as a fundamental unit of memory storage. Understanding the mechanics of assembly formation is needed to link the biology of the nervous system to its function. In previous models, assemblies have formed from unsupervised learning of sensory stimuli via synaptic plasticity

However, learning certainly depends on supervisory signals such as reward. While reward-based learning is widely used in machine learning applications, like reinforcement learning it is not clear how it is incorporated in biological circuits. In addition, unpublished data from the Oswald lab challenges the current working model of assembly learning: They present two odors to freely behaving mice, one rewarded and one unrewarded. Based on a combination of activity-dependent labeling, optogenetic stimulation, and in vitro (soon in vivo) recordings of neurons in the piriform cortex they find that, unintuitively, an assembly only forms for neurons selective to the rewarded odor.

But how is reward-based learning implemented in a biological circuit? Published data from the Oswald lab suggests that activation of VIP inhibitory neurons (INs) can gate the synaptic plasticity essential for assembly formation (Canto-Bustos et al., 2022). Therefore, the following hypothesis of reward-based assembly learning emerges: Presenting an odor together with a reward activates VIP neurons, which gates synaptic plasticity among odor-specific PNs.

Based on a close collaboration with the Oswald lab, my host supervisor's experience in modeling circuits with multiple INs, and my prior knowledge in modeling plastic excitatory/inhibitory networks, I aim to uncover how reward-based learning is implemented in biologically plausible circuits. First, I aim to build an experimentally constrained computational model of the piriform cortex circuitry, including PNs and the major INs (PV, SST, and VIP) with plastic synapses, to test the above-described hypothesis of reward-based assembly learning gated via VIP neurons. Second, I aim to show that only rewarded odor-specific PNs form an assembly; here preliminary data suggest that SSTs play a pivot role. Third, I aim to extend the model to allow for encoding of multiple odor assemblies with overlapping chemical components in order to study how multiple components are combined to form a meaningful odor perception.

My work will lead to a better understanding of olfactory processing; how cortical circuits are able to support reward-based learning paradigms; the biological basis of understanding more complex reward-based learning paradigms (e.g., reinforcement learning). I hope that my results can be generalized for application to other brain regions involved with associative learning problems, such as the hippocampus.

06 Neurobiology and Intelligence

Role of Visual and Emotion Circuits in Shaping Neural Spatial Representations of Aversive Stimuli

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In order to survive, animals navigate the environment to find food, shelter, and avoid predators. The development of internal spatial maps is believed to facilitate these tasks with visual inputs providing critical information on the position and dynamics of external stimuli. These maps, found in the hippocampal and parahippocampal formations, are thought to help animals to define their position in the environment and locate other members of the same species. However, little is known about the neural networks that support spatial representations of explicitly dangerous and threatening visual stimuli, such as predators.

This project aims to dissect the circuits that enable spatial representations of aversive visual stimuli and their association with emotional content across the visual and spatial navigation systems. Two main systems process visual information: the geniculate and collicular pathways. The geniculate pathway transmits visual input to the primary visual cortex (V1), which extracts basic features from the visual scene. Intriguingly, clinically blind patients with complete V1 lesions are still able to navigate and react to threatening visual stimuli. This phenomenon, referred to as «blindsight,» has been linked to the collicular pathway. The collicular system relays object motion information from the superior colliculus, an evolutionary ancient visual center to the postrhinal cortex (POR), which is considered one of the main entry points of visual input to the hippocampus. In addition, POR is involved in contextual fear conditioning and is the only visual cortex reciprocally linked to the amygdala, a crucial structure for aversive information processing.

For these reasons, we hypothesize that POR is critical to developing spatial representations of aversive stimuli by coupling object movement information, stimulus value, and animal self-position. By identifying salient and dangerous visual stimuli, POR would allow an animal to maintain an updated representation of the environment and to adapt its behavior when changes occur. Through behavioral, electrophysiological, imaging, and optogenetic approaches in freely moving mice, I propose several studies. First, record hippocampal and parahippocampal responses to moving visual stimuli and optogenetically silence the geniculate and collicular pathways to determine their contributions to spatial representations of moving objects. Second, determine the influence of emotional content on the spatial representation of moving objects by combining these stimuli with aversive ones. Third, disentangle the circuits involved by focusing on amygdala/POR reciprocal connections.

I will manipulate these projections to test their role in shaping the spatial representations of moving visual stimuli in fear learning paradigms. Overall, this project will provide crucial insight into how the visual and navigation systems process threatening stimuli, such as predators, to generate complex behaviors essential for survival.

Understanding the Formation of Schema Representations in Prefrontal Cortex

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Humans and animals learn schemas – situational learning derived from prior experiences – and apply them to novel problems to efficiently guide their decisions. Prefrontal cortex (PFC) has been identified as a key brain area that encodes schemas. However, how such schemas are implemented in neural circuits is unclear, yet fundamental, to a mechanistic understanding of flexible behavior. Emerging research in the host laboratories has begun to provide insight into this question; in a sequence learning task in mice, the team revealed how PFC neurons encode the learned schema and map it to new tasks. Critically, the data suggest a mechanism for building the schema representation that, if true, would generalize to complex real-life schemata and reveal a novel, general mechanism for structuring behavior. Testing this theory requires tracking individual neurons in PFC from the onset of learning to expert behavior, which is one of the skills I developed during my doctoral work.

In this project, I aim to characterize how prefrontal cortex forms schema representations with learning. First, I will develop a head-fixed version of the sequence learning task to enable simultaneous two-photon calcium imaging. I will use this design to follow the activity of PFC neurons in task across learning to identify how and when the schema representation arises. Finally, I will investigate the role of inputs from key areas including entorhinal cortex (EC) and hippocampus (HC) and dopamine signals in the formation of schema representations.

I hypothesize that three steps are involved here. At the start of learning, PFC neurons map progress through individual goal-directed actions (termed phase cells). Then, these cells split into groups tracking the progress of each action in the schema (i.e., subgoals), allowing generalization across tasks. Finally, this learning and subsequent generalization relies on inputs from EC and HC (providing schema content), and dopamine signals (enabling remapping). I will use virtual reality technology to create a spatial sequence learning task for mice that are head-fixed on a track ball. I will then make medial PFC accessible for two-photon imaging with a microprism implant and transduce neurons in PFC with a fluorescent calcium indicator. This will enable chronic recording of cellular and subcellular calcium activity in behaving animals throughout learning. Next, two-photon imaging of a dopamine sensor will allow me to follow dopamine signals in PFC during learning and generalization. Lastly, by expressing a calcium indicator in EC and HC neurons, I will image their axon terminals in PFC and record the information communicated to PFC before, during, and after learning.

I plan to observe what inputs PFC neurons integrate during learning to elucidate what changes in neuronal computations lead to schema representations. My project will shed light on how prefrontal cortex forms models of tasks to efficiently and flexibly guide behavior in novel situations.

Neural Mechanisms for Dynamic Action Planning and Execution as We Move Through Space

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Our understanding of the neural mechanisms underlying voluntary behavior comes primarily from experiments conducted under simplified laboratory conditions. For example, reaching has been studied mainly in seated subjects making arm movements with their body stationary. While the human neuroimaging studies conducted during my doctoral studies contributed to this body of work, I have also become increasingly aware of its limitations. In particular, it is unclear how well the insights of this approach will generalize to the more complex scenarios encountered during natural behavior, in which we plan and perform actions in a dynamically changing environment as we move our body through space. Understanding this is crucial for new theories of brain function and clinical applications that better translate to the real world.

I propose to pursue a more naturalistic approach to studying voluntary movement. I will bring my expertise on frontoparietal interactions during online reach control in body-stationary human subjects to the design of new neurophysiological experiments in non-human primates (NHP) that investigate the neural mechanisms of voluntary reaching during body motion. These experiments will be conducted in the laboratories of Andrea Green and Paul Cisek, experts in dynamic sensorimotor control and action selection in NHPs. I will perform simultaneous multi-electrode recordings in multiple areas related to reach control (primary motor, dorsal premotor, posterior parietal cortex), allowing me to examine for the first time the activity of individual neurons and the dynamics of neural populations as monkeys select, plan, and execute reaching movements while being moved around in space by a unique 3D motion system.

One goal will be to extend current motor control theories by examining how control policies are adjusted to the changing spatial relationships and reach dynamics that arise as the body moves in space. I will test the hypothesis that cortical reach circuits process body motion information in a sophisticated way, taking into account both body-limb biomechanical interactions and the context of the behavioral goal. I will also study the mechanisms that keep track of the location of potential reach goals and select between them as the body moves during planning. I will test the hypothesis that as the body moves the spatial locations of reach goals and the competition between potential actions are updated continuously within a distributed representation of reachable space.

This project will move my research in a fundamentally new direction, placing me in a unique position to push theories of cognition and action towards the more dynamic scenarios we experience daily during natural behavior, with important implications for rehabilitation and neuroprosthetics. Overall, it will help launch an independent research program that shifts current experimental paradigms toward a neuroscience of natural behavior.

Genetically Encoded Voltage Indicators to Uncover the Role of Golgi Cells in Pattern Classification

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Whether a surgeon is performing a complex procedure, or a farmer is lifting a box of vegetables, humans rely on the cerebellum to refine our movements. Yet, the cellular mechanisms underlying the encoding and recognition of sensorimotor sequences within the cerebellar cortex are not well understood. Classical theories propose that sparse activation of Granule Cells (GrCs) is essential for pattern classi-fication of sensorimotor information coming through Mossy Fibers (MFs). Recent in vivo recordings reveal extensive activity of GrCs, which challenges the current understanding of cerebellar function.

My hypothesis is that inhibitory Golgi Cells (GoCs) play a key role in shaping patterns of GrC activity by encoding relevant behavioral information. GoCs provide a major source of inhibitory input to GrCs and have extensive connectivity with synaptic inputs from MFs and Parallel Fibers (GrCs' axons), re-entry loops from Purkinje Cells (via inhibitory Lugaro Cells) and inhibitory neurons from Deep Cerebellar Nuclei (DCN), as well as spillover-mediated input from Climbing Fibers (CFs). Paired patch-clamp recordings and computational simulations suggest that electrical coupling between GoCs may enable them to both synchronize and desynchronize during behavior, thus, adding more complexity to the system.

I will study the functional relevance of these connections to GoCs, using the Genetically Encoded Voltage Indicator (GEVI) JEDI-2P, during postural adjustments of head-fixed mice, a cerebellar-relevant behavior. The host lab has developed viral expression strategies and custom-made multiphoton optical technologies to optimize GEVI recordings, suited to record the fast kinetics of GoCs spikes and their subthreshold activity, as well. In particular, the combination of GEVI and Ultrafast Local Volume Excitation, in a random access microscope, enables to overcome major limitations inherent to all-optical interrogations of neuronal circuits in behaving animals.

My project will address the following questions. First, do GoCs population encode specific sensorimotor features or the general behavioral context? Second, how plastic is the functional extent of electrical connectivity between GoCs? Third, are there clusters of GoCs shaped by CFs spillover? Four, how do re-entry loops participate in shaping GoCs activity? Depending on the question being investigated, a red-shifted Genetically Encoded Calcium Indicator will also be expressed by MFs or GrCs (Q1), CFs (Q3), Lugaro cells or DCN neurons (Q4). I will use optogenetic stimulation to measure electrical coupling in vivo for Q2. Computational simulations might also be performed. This project will provide insights into the dynamics of inhibitory patterning and the role of electrical coupling in the mammalian brain, during circuit-specific relevant behavior with unprecedented spatial and temporal resolution.

The Neuronal Mechanisms Underlying State-dependent Odor Preference

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Internal states, such as hunger and thirst, are strong motivational forces that drive us to pursue goals and meet our basic needs. To induce adaptive behavior, specific sensory information needs to be integrated with the internal cues regarding the state of the animal.

For example, in my host laboratory mice exhibited hunger-dependent food odor preference, which involves neuropeptide Y (NPY) release in the paraventricular thalamus (PVT) from hypothalamic hunger neurons (AGRP neurons). The PVT is a midline thalamic structure that plays a vital role in various motivated behaviors and is also associated with arousal and valence processing. The PVT is highly interconnected, receives inputs from multiple brain regions, including the hypothalamus and the brainstem, and has reciprocal connections with several cortical regions. This extensive anatomical connectivity underscores the potential role of the PVT as a hub for linking internal states and external information to drive behavior. Importantly, the PVT consists of heterogeneous cell populations with subgroups of neurons showing distinct connectivity and transcriptomes.

In this project, we will investigate neuronal mechanisms within the PVT that drive odor-preference behavior under various internal states. We anticipate that our findings will contribute to a better understanding of the neural circuits and mechanisms underlying the interaction between internal state and external sensory cues.

Vocal Communication and the Evolution of Hyper-cooperative Societies

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Humans are "hyper-cooperative:" we regularly engage in long-term and strategic cooperation with non-kin. This propensity for working together has allowed us to dominate the planet, and the key to our success is language. However, humans are not the only animals capable of hyper-cooperation. In Shark Bay, Australia, unrelated male bottlenose dolphins work together in an extensive network of nested alliances — 'partners' in 'teams' in 'clubs' — to control reproductive access to females. Given that dolphins also display remarkable vocal complexity via diverse repertoires of whistles, is vocal communication the mechanism behind hyper-cooperation in this society as well? Or did dolphins evolve a different way of building and maintaining hyper-cooperative relationships?

I aim to discover how the rare, but powerful, phenomenon of hyper-cooperation unfolds in an unconventional, underwater study system. I hypothesize that dolphins, like humans, rely on vocal communication to mediate the interactions underlying their hyper-cooperative society. I predict this will be accomplished through alliance-specific whistle repertoires.

First, I will quantify similarities and differences in whistle usage among many alliances. Second, I will evaluate what specific vocalizations mean by playing different whistles to individuals and recording their behavioral and acoustic responses in the field (via drone video footage and underwater microphones, respectively). Third, I will use social and behavioral datasets spanning more than 40 years to determine whether variations in whistle usage impact long-term fitness (e.g., number of paternities, female consortship rates) of individuals and alliances. Finally, I will build mechanistic agent-based models to explore the interplay between vocal communication and hyper-cooperation over evolutionary timescales. The models' agents will be parameterized to behave like real-world male dolphins. The emergent property will be social network structure (i.e., nested alliances). The manipulated condition will be whether agents can vocally signal, and the form that such signaling takes (i.e., able to signal to single individuals vs. groups). Comparative research is crucial to understand how and why hyper-cooperation has so rarely evolved. The alliances of male dolphins in Shark Bay are the largest and most complex hyper-cooperative network known beyond humans.

Supported by 40 years of research, these dolphins offer an exceptional opportunity to understand the vocal mechanisms that underpin hyper-cooperative behaviors. This ambitious project synergizes my quantitative skills, Dr. King's expertise in dolphin communication, and the University of Bristol's research facilities, enabling me to move into exciting and challenging fields (e.g., playbacks, modelling). I will answer unresolved, big-picture questions about the role of vocal communication in the evolution and persistence of hyper-cooperative societies.

Neural Dynamics of Social Role-taking and Role-reversal in the Medial Prefrontal Cortex

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Most current knowledge about the neural representations of social role-taking stems from the study of dominance-subordination hierarchies, mostly in the tube test. This knowledge has only recently expanded to different social contexts with the rat-human version of the game 'hide and seek.' Unlike social status, social play tends to be pleasurable for all individuals involved and relies more on cooperative behaviors that allow animals to pretend to occupy roles that they usually do not. Despite these differences in the social context and in the freedom animals have to reverse roles, recent studies of the medial prefrontal cortex (mPFC) activity hint at the existence of a common neural code that pervades social roles in all contexts. Anecdotal evidence from these studies suggests that, although mPFC neurons encode specific behaviors in the test tube and in hide and seek, respectively, subsets of these neurons may underlie the abstract representation of social role itself.

For example, in hide and seek, as rats learn that the opening of the starting box indicates they are to be hiders (open) or seekers (closed), nearly 30% of mPFC neurons exhibit responses to the starting box closure during "seek" trials that are not explained by other variables, which suggests an influence on role definition. The current understanding of the high-level representation of social roles (i.e., independent of specific social contexts) is limited to a few results and has not been systematically addressed.

The goal of this project is to study the neuronal representation of social roles across role-taking situations (i.e., playful or not) to disentangle the common neural code that may underlie them. The hypothesis is that abstract features associated with similar roles (i.e., dominance-seeking, subordinance-hiding) are redundantly encoded by mPFC neuronal subsets. Specifically, we aim to explore the high-dimensional neuronal space for common codes between tasks; identify role-defining neurons to study their dynamics during task transitions and role-reversal; use decoding tools to test the hypothesized code redundancies for similar roles; and optogenetically manipulate role-taking by targeting mPFC neurons.

To this end, Long-Evans rats will be chronically implanted with multiple-shank Neuropixels 2.0 or optic fibers in the Cingulate, Prelimbic and Infralimbic cortices, trained to play hide and seek and tested in the test tube to identify social ranks. Contrary to previous studies, this will allow the trial-by-trial simultaneous recording of hundreds of units across cortices and layers in the same animal and the comparison of activity across tasks and neural subpopulations. Unlike other role plays (e.g., play fighting), hide and seek does not involve direct aggression that could damage implants or produce confounding data related to effortful behaviors. Overall, this project will start to fill the gap in our knowledge of the neural underpinnings of social roles and the cortical dynamics by which they are adopted and reversed.

Closing the Neural and Behavioral Feedback Loop of Social Interactions in Freely Behaving Primates

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I aspire to study the neural basis of social cognition, especially the processes that implement Theory of Mind in humans, which likely exist in other species to varying extents. Theory of Mind is the ability to put oneself in someone else's shoes. It lets us adapt our behavior based on norms, read between lines and keep unity in a complex society. People with autism spectrum disorder often struggle with this, which may lead to constant social stress and higher comorbidity risks. Thus, understanding the neural basis of social cognition is imperative.

I will study the neurocognitive processes of social decisions in rhesus macaques using an innovative closed-loop approach in free-foraging individuals. Macaques are evolutionary much closer to humans than rodents and the best studied species for higher neurocognitive functions, which makes them the most advanced animal model for theoretical and clinical neuroscience. Still, to what extent their cooperative behavior compares to that of humans remains debated. Also, due to technical limitations, most neurophysiological data come from experiments using highly restrained behaviors. In contrast, natural social interactions involve dynamic, mutual exchanges between agents. I aim to fill these gaps by recording neural data from macaque pairs during free social foraging in a novel and unique environment with multiple experimentally controlled dyadic feeders.

I hypothesize that for macaques to behave cooperatively there must be dynamic interactions between the foraging reward distribution and the pairs' social and spatial states (e.g., hierarchy, past interactions, how far the feeders/peers are). I predict the level of cooperation will vary based on these states and reward distribution and the neural activity in social, spatial, and reward-oriented brain areas will distinctly reflect respective states.

My approach will combine wireless neural recording, AI-based action recognition, and cortical microstimulation to analyze and causally manipulate the activity of target areas and behavior. I expect to establish the link between cingulate areas and social states, retrosplenial cortex and spatial states, and prefrontal areas and reward states and to test their causality by stimulating one area while recording in the rest. My project will explore the fundamental, yet previously inaccessible, decision variables in an ethologically relevant setup with rigorous experimental control, closing the loop of brain and behavior as an organic unit.

The Role of Simultaneous Population Dynamics in Associative Learning

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Associative learning, in which a new response becomes associated with a particular stimulus, is a vital cognitive process in our daily functioning, as it allows us to acquire new information and to constantly adapt to changes in our surroundings. Although associative learning was repeatedly shown to restructure the activity of numerous individual neurons across cortical and subcortical regions, it is still not clear how it is represented at the population level. Specifically, how learning-related activity patterns develop in single brain areas, how interregional interactions support it, and how these widespread representations flexibly update as stimuli-outcome contingencies change.

My project will focus on the population dynamics underlying associative learning by combining behavior, population recordings, and a dynamical systems-based analytical framework. The prefrontal cortex (PFC) and the basolateral Amygdala (BLA) are two extensively interconnected learning hubs and they offer an ideal anatomical infrastructure to investigate inter-regional communication effects on local learning-related activity patterns. I plan to harness the cortico-amygdala circuitry and use Neuropixel probes to concomitantly record population activity in PFC and BLA as mice perform appetitive associative reversal learning tasks, in which the cue-reward contingency is swiftly reversed after they have slowly learned the original contingency. In this way, learning will be studied on multiple timescales, from a few minutes to whole days. Next, I will use population decoding to identify the neural correlates of the paradigm in PFC and BLA, examine the temporal interplay between them, and investigate how information routing supports population activity and consequently shapes learning. Lastly, I will examine the causality between interregional communication, local task-related representations and behavior by optogenetically manipulating the Amygdala-cortical projections.

I propose a novel hypothesis, in which the formation of cue-outcome contingencies is paralleled with the development of distinct sequences of neurophysiological signatures both at the PFC and the BLA. These local signatures will co-evolve through inter-regional communication to mediate the selection and stability of stimuli-outcome contingencies with varying time constants that will determine individual learning rates. As the BLA is typically known to process salient information, such as emotion, valence, or motivation, I further suggest that it is the first to detect the positively valenced stimulus and then to instruct the PFC to initiate learning via command signals routing from the BLA to the PFC. Such comprehensive mechanistic investigation of how PFC-BLA population dynamics underlie associative learning, on multiple timescales, can reveal general principles of how the brain utilizes inter-regional interactions to learn associations and ultimately, shape behavior.

The Neural Basis of Naturalistic Memory

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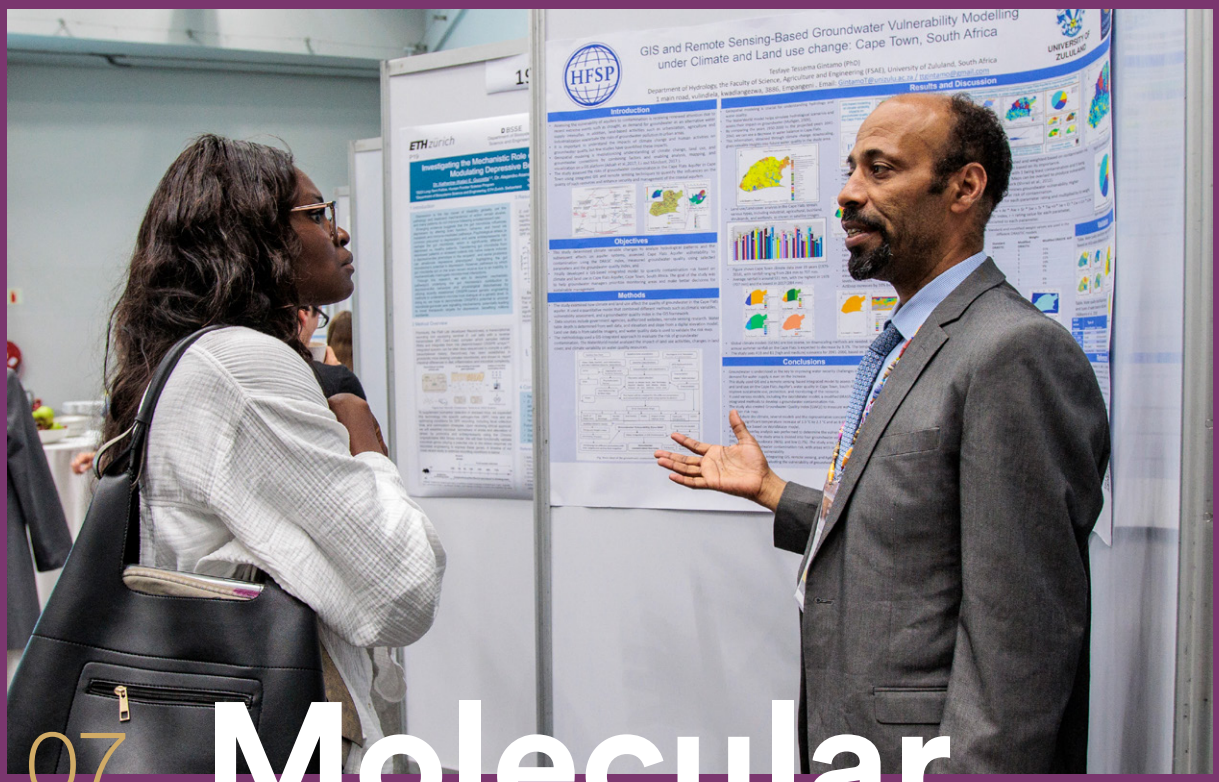
Memory impairments are extremely common as part of healthy aging, but we still lack an understanding of how experiences are remembered, or why this process can so often fail. Possible breakthroughs have been hindered by two major obstacles: the use of coarse neuroimaging tools that measure activity averaged across large neural populations and the use of highly simplified experimental paradigms. These practices have uncovered simple features of how memories are formed, but cannot capture critical features of real-life experiences. Realistic life experiences consist of a continuous stream of information that is connected from moment to moment, but how do memories form amidst continuous stimuli fluctuations? How do we naturally make choices about segmenting this experience into individual events and how can we later remember them? Unfortunately, most of the detailed neural mechanisms underlying these processes are still unknown. Rodent studies have suggested potential mechanisms, but cannot capture the full complexity of human behavior.

In this project, I propose a unique experimental approach that will provide the first evidence in humans for the mechanisms by which streams of sensory experiences are segmented and stored by the human brain as discrete memories. We will collect a dataset using intracranial electrodes to measure the activity of individual neurons, a type of recording that was not possible in humans until recently. This will allow us to study the precise firing of neurons during the creation of a memory trace and test competing hypotheses about detailed neural activity.

We expect neurons to build up an event representation by rapidly replaying a sequence of information from that event, multiple times a second. To explore how that sequencing takes place we will examine the time, frequency, and phase domain of the signal using recently developed tools (e.g., phase-amplitude coupling and circular statistics). Participants will be asked to perform a task in which they must watch a series of short movies and then perform a cued-recall task. By testing memory for extended experiences, such as movies, participants will be required to build up complex models of the event unfolding in front of them over time, which parallels the demands the brain goes through while encoding real-life experiences.

We hypothesize that mechanisms extensively observed in rodents, such as theta cycle resets (4-8 Hz oscillations) and sharp-wave ripples (strong ~150-Hz oscillations), will support the above-mentioned sequencing of information allowing the brain to encode/recall a new event. I look forward to collaborating with the host supervisor, who is one of the few researchers in the world with access to this unique type of human neural recording and the quantitative expertise necessary to analyze it.



07

Molecular Basis of Complex Diseases

Deciphering Genomic Determinants of Immunity in Tumor Organoid Models at Single-cell Resolution

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Cancers are complex cellular ecosystems with stromal, immune, and tumor cell components. Large-scale genomic analyses and single-cell atlases have revealed the immune composition across tumors, but only capture a snapshot of the dynamic tumor-immune interface. How the different cell types and states in a tumor dynamically respond to immune challenge, and how variation in the responses is defined by tissue type, somatic genetic and interindividual variation remains currently unexplored. This is an important open question, as the cellular interactions represent druggable molecular targets.

Experimental models where cancer cells are challenged with immune attack are needed to understand the dynamic responses. Existing models fail to recapitulate the phenotypic and genetic complexity of patient tumors, relying on two-dimensional clonal cell lines, single immune cell types, and readouts with insufficient resolution to capture the various differentiation states and genetic subclones. New technologies such as advances in organoid culturing and CRISPR screening combined with single-cell multi-omics harbor the promise of deciphering these cell circuits in a high-throughput framework.

I aim to use patient-derived tumor organoids to experimentally reconstruct the solid tumor immune microenvironment, and investigate genomic and cell type determinants of tumor-immune interactions using genetic perturbation screens with a single-cell multi-omic readout. I hypothesize that cancer cells from different tissues, harboring different genetic alterations, or cancer cells in distinct differentiation states within the same tumor respond differently to immune challenge. Furthermore, cancer cell epigenetic states underlie such heterogeneity, and understanding these mechanisms will reveal new paradigms of immune regulation in both malignant and healthy tissues.

Using over 250 organoids from solid tumors including colorectal, pancreatic, esophageal, and ovarian cancer, I will examine the single-cell gene expression and chromatin accessibility of tumor cells in response to various cell types of the immune microenvironment. Integration of these responses with tumor genetic alterations, including at subclonal levels will reveal genomic determinants of variation in immune reactivity. To dissect the mechanisms driving the transcriptomic responses, I will use single-cell multi-omic CRISPR screens to silence transcriptional effectors and regulatory elements and engineer precise alterations using base editing. These approaches build on the strengths of Dr. Garnett's laboratory, including a biobank of over 250 tumor organoids and CRISPR knockout and base editing screens, and my previous experience in immunology of blood cancers.

The study will open previously unexplored directions into determinants of immunity in cancer and regulation of cell-type specific immune responses beyond cancer.

Unraveling Spatial Landscape of Mycobacterium Tuberculosis Transcriptome and its Role in TB Pathology

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Despite the advent of vaccination and antibiotics, the global burden of tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) remains high. Previously regarded as a non-compartmentalized cellular system due to a lack of well-defined intracellular organelles, recent studies have shown Mycobacteria possess defined cellular organization with distinctive spatiotemporal localization of proteins. In eukaryotes, it is well reported that targeting mRNA transcript to specific cellular locations is critical for spatial protein synthesis, thereby highlighting RNA localization as a driver of sub-cellular organization. Fascinatingly, only recently has the similar phenomenon of RNA localization been demonstrated in bacteria like E. coli. Bacterial mRNA can localize to nucleoid, cytoplasm, poles, and inner membrane. However, the underlying molecular mechanisms dictating their localization are not completely understood. The transcriptome organization of Mtb remains elusive so far. Moreover, the biological relevance of defined RNA localization patterns in bacteria needs to be ascertained.

My hypothesis is that RNA localization in eukaryotes is an important post-transcriptional regulatory mechanism. However, the scope of RNA localization in pathogens like Mtb remains unknown; the Mtb transcriptome might be spatiotemporally organized to maximize gene function and to provide fast, efficient regulation in response to changing host environmental stimuli. I will pursue three main goals. First, delineate spatiotemporal organization of the Mtb transcriptome under homeostasis and assess if there is reconfiguration in response to different physiological stresses. Second, characterize molecular mechanisms driving Mtb RNA localization and enabling tethering to specified location. Third, investigate whether spatial information regulates transcriptional activity and establish whether disruption of spatial organization introduces vulnerabilities in Mtb growth and survival.

I will employ methods recently developed in the Rubin lab to perform fluorescence in situ localization (FISH) to localize transcripts in viable Mtb and to recreate pseudotemporal localization in fixed bacteria. We will use cellular fractionation and RNA sequencing techniques to spatially map the complete Mtb transcriptome and immunoprecipitation studies to identify any interacting motor and cytoskeleton proteins. I will define sequence determinants that could direct transcripts to subcellular compartments and identify proteins that might play a role in localization. Finally, I will generate mutants that mislocalize RNAs and determine their physiological effects.

RNA localization adds a layer of complexity to prokaryotic cellular architecture. Understanding the physiological relevance of such dynamic spatial organization of molecular components, especially in clinically relevant pathogens like Mtb, might provide a new paradigm of gene regulation and function that can be therapeutically exploited in future.

Unmasking the Role of Nuclear Antioxidant Systems in Human Disease

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Human metabolism heavily relies on oxygen (O2) as a primary electron acceptor in around 150 metabolic reactions, essential for many processes, such as mitochondrial respiration. However, the involvement of O2 in metabolism can lead to the generation of reactive oxygen species (ROS), unwanted molecules that have the potential to oxidize and damage various cellular components, including nucleotides, proteins, and lipids. To counteract the harmful effects of ROS, aerobic organisms have developed antioxidant systems, primarily localized within the subcellular spaces where ROS-generating enzymes are prevalent. Mitochondria, being a major source of cellular ROS, possess specific isoforms of enzymes that help dissipate ROS, or repair their damage. While the nucleus is a metabolically active organelle engaged in DNA replication, transcription, and gene regulation, it is also susceptible to ROS generation due to the activity of two enzyme families involved in demethylation and hydroxylation reactions, both of which require O2. However, it is unknown whether the nucleus has dedicated antioxidant systems to protect against ROS toxicity and maintain nuclear functionality.

I hypothesize that cells have developed mechanisms to inhibit nuclear ROS and preserve the integrity of DNA and the nuclear envelope. Existing approaches lack the temporal and spatial resolution required to test this hypothesis. Therefore, I aim to develop molecular tools to enable the systematic discovery of nuclear antioxidant systems and their significance in human physiology. Aim 1: Investigate the mechanisms involved in dissipating nuclear ROS. To explore nuclear antioxidant pathways, we will employ an optogenetic approach to induce high levels of ROS using a flavoprotein localized to the nucleus. This will allow us to assess canonical cell survival mechanisms and perform genetic screens using a nuclear-focused library. Aim 2: Discover redox-sensitive nodes in the nucleus. Given that both enzyme families involved in nuclear ROS generation are fueled by alpha-ketoglutarate and inhibited by succinate, we will manipulate ROS production by supplementing cells with permeable forms of these. I will develop a rapid nuclei immunopurification system and couple it to mass spectrometry analysis. This approach will identify ROS-sensitive alterations in proteins and lipids. Aim 3: Investigate the connection between nuclear ROS and DNA leakage.

I hypothesize that ROS may compromise nuclear envelope integrity, thus, leading to DNA leakage, a type of stress sensed by cGAS, which ultimately triggers cell intrinsic and extrinsic immunity. Using the aforementioned tools, we will modulate nuclear ROS, evaluate cGAS activation, and analyze the tumor response to immune-mediated growth arrest and checkpoint therapy. Overall, this project aims to develop innovative technologies to uncover the unexplored role of ROS in the nucleus and its potential association with disease states related to DNA stress.

This project will represent a breakthrough in understanding the molecular basis of mechano-epigenetic memories and may open avenues for designing new strategies to erase maladaptive memories to drive scarless tissue regeneration.

08

Microbes and Their Adaptations to Their Environments

Microbial Consortia with Direct Electron Transfer: in situ Visualization of Community Robustness

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Multi-species consortia of microbes shape human health and environment. Their chemical output emerges from the specialized metabolism of consortia members and the interactions between cells in a close, physical grid. Thanks to recent discoveries, we now know that microbial consortia in anaerobic conditions can use electricity to exchange energy between cells. The direct transfer of electric current by nanowires and cytochromes provides these consortia with a low-loss, high-specificity mechanism of exchange that spans larger distances than the exchange of diffusible compounds. This way, direct electron transfer has emerged as a fundamentally different mode of interaction in microbial ecology. Current research focuses on the molecular mechanism of electron transfer and the spatial patterns of cell activity at steady state. How such sophisticated interaction networks respond to an environmental perturbation remains an open question. Importantly, this response cannot be predicted from the principles that currently exist.

My aim is to address this question directly for methane-metabolizing communities, by measuring the response to nutrient concentration both at the level of the population, as well as for single cells. To do so, I will leverage the expertise of the sponsor lab to observe single cells responding to nutrient pulses in their original aggregate structure. I will connect these two levels of observations with a quantitative, predictive modeling framework to build on my previous theory work on nutrient limitation.

I hypothesize that the response to environmental perturbation emerges in two layers. Firstly, the spatial structure of the aggregate defines a base access of each cell to electron transfer with the partner species. Secondly, cells may experience limitation for diffusible nutrients like nitrogen or vitamins, especially if they are close to a partner cell and the base limitation from electron transfer is relatively weak. To resolve the environmental perturbation in a spatial setting, we need to quantify the relative strength of response for a set of nutrients and do so for single cells.

I will test these hypotheses in a naturally evolved system of direct electron transfer, specifically, the granular aggregates of anaerobic methanotrophic archaea (ANME) and their sulfate-reducing bacterial partners (SRB) that work together in the deep-sea environment to remove 80% of all methane released from the seafloor. I will apply a nutrient-pulse with stable isotope tracers to map the single cell response within aggregates using the analytical imaging techniques from the sponsor lab (FISH-nanoSIMS).

The project will test the impact of direct electron transfer on community response in an environmentally relevant microbial consortium, enable me to transition from theory to experiments, and provide analytical tools to visualize the cell response in a large range of structured microbial aggregates for future research.

Uncovering the Secret World of Microbial Enzymatic Decorated Nanowires

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Acetogenic bacteria subsist at the thermodynamic limit of life under extremes of temperature, salinity, food scarcity, and anaerobic environments. They utilize the conserved, phylogenetically ancient Wood-Ljungdahl metabolic pathway (WLP), in which hydrogen is the electron donor and carbon dioxide is the terminal electron acceptor. Their comparatively alien metabolic machinery utilizes redox-reactions in which molecular machines employ metalloclusters for electron transfer and act as catalytic centers. Through our active research on modularity, where domains act in electron donor modules, while others transmit electrons to catalytic acceptor modules, the Schuller Lab discovered hydrogen dependent CO2 reductase (HDCR), the first enzyme of the methyl branch in the WLP from *Thermoanaerobacter kivui*, forms enzymatic, protein-decorated nanowires. Filamentation and modularity increase enzyme stability, which explains HDCR's unsurpassed catalytic activity. HDCR catalyzes formate synthesis from CO2 a 1000-fold more effectively than chemical catalysts by relying on iron-sulfur cluster networks created by nanowire formation. HDCR filaments bundle in cells, forming ring structures attached to the plasma membrane – an untapped discovery with functional and physiological implications.

We aim to understand membrane anchoring mechanisms and the ring structures' biological role. We hypothesize this is an adaptation to low CO2 levels, acting as a carbon concentration mechanism, inducing efficient CO2 capture and utilization. Identification of proteins in HDCR-membrane interactions will be performed through in vivo protein crosslinking paired with genetic manipulation and cryoET complemented by in vitro assays. We hypothesize this effective architecture is not unique and diverse, biological nanowires are ubiquitous in nature.

We identified the *Aromatoleum aromaticum* AOR enzyme as an HDCR-type nanowire (Winiarska et al. 2023), which indicates more biological nanowires are awaiting discovery. Our work will demonstrate that nanowires are crucial adaptations for anaerobic life to respond to stress and endure environmental extremes. Our aim is to identify new biological nanowires through collaboration with Dr. Georg Hochberg, a local expert in phylogenetic and evolutionary biochemistry. We anticipate discovering new nanowires through data mining, phylogenetic analysis, and protein-structure prediction methods. After identification, organism cultivation using our lab's resources, native isolation, enzymatic character-ization, and cryoEM will be achieved with our established biophysics and biochemistry pipelines.

We hope to discover new modular redox functions linked to nanowire cores and elucidate their forma-tion, illuminating how anaerobes adapt and survive at the extremes of life. Novel redox functions and nanowires can be adapted for carbon capture and biotechnology applications, which will address industrial and environmental needs while expanding the synthetic chemistry toolkit's reach for reduction reactions.

Mapping the Interactions Between Bacteria in the Microbiome for Targeted Strain Replacement

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The importance of the human gut microbiome in health and disease highlights the need for ways to actively modulate it. Although fecal microbiota transplantation is used to profoundly change dysbiotic microbiotas, more targeted and rational ways of modulating the microbiome are currently missing. Replacing a specific strain by another strain of the same species that carries a desired trait for the community is a strategy that would ensure minimal impact on the community composition as a whole. To be able to do this and overcome the colonization resistance of such stable microbial communities, we need to understand the underlying community dynamics.

I propose using two of the most abundant and prevalent species in the human gut microbiota, *Bacteroides uniformis* and *Phocaeicola vulgatus* and coming up with strategies for strain replacement. My research will include four aims. First, identify *B. uniformis* or *P. vulgatus* strains that kill the resident co-specific strain of five characterized human gut microbiotas. Second, study whether and how the microbiome protects a resident strain from inhibition by the incoming strain. Third, for interactions that persist at the community level, dissect the mechanistic and genetic basis of the interaction. Fourth, introduce the killing genetic trait and an additional desired trait into the resident strain and use this modified strain for strain replacement.

With this project, I am working with multiple hypotheses. First, I suspect different strains of the same species commonly interact via negative interactions. I expect to identify mainly cases of intraspecies inhibition, and in some cases the identified inhibition interactions will also occur when the strains are in a complex community setting, whereas in other cases, the community may protect the resident strain. Second, I hypothesize that for some of the identified interactions, a specific gene or group of genes will be necessary and sufficient for inhibition\protection of a strain from inhibition, and thus, can be identified by genetic screens and introduced to other strains if necessary.

My project will work with existing large strain collections of *B. uniformis* and *P. vulgatus* to test pairwise interactions within each species. Negative interactions will be checked for their persistence within microbiome communities using fluorescence tagging, or plating on selective plates to monitor the two strains of interest. Mutant libraries and follow-up targeted experiments will be used to decipher the genetic basis for these interactions. Personalized human microbiomes, for which the individual species/strains have been isolated, will be used to identify community protection mechanisms.

This project utilizes my skills in studying interactions between microorganisms and the Typas laboratory's systematic screening and microbiome expertise. This study will lead to new discoveries regarding how bacteria interact in natural communities and provide important mechanistic understanding to enable successful strain replacement in gut microbiomes.

09

Microbes, Viruses, Parasites, and Their Hosts

Parasite Immune Evasion in a Murine Microcosm of the Human Immune System

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Hosts and parasites exist in a delicate balance: many of these associations are ancient, the result of an equilibrium of adaptation and counter-adaptation as described by the Red Queen's Race hypothesis. The host-parasite interface is, thus, the site of rapid evolution and of nature's most elaborate weapons and ruses. The shapeshifting of *Plasmodium falciparum*, the most lethal malaria parasite, provides an example. Infection with sporozoites (PfSPZ), the stage of which moves from bite through blood to the liver, does not induce sterilizing immunity. Vaccines and therapeutics for this stage must contend with its immune evasion. PfSPZ's surface is heavily covered with circumsporozoite protein (PfCSP), which is critical for mammalian host cell invasion. PfCSP includes a central region comprised of repeating tetrapeptides. These copious repeats have been proposed as an immune-evasive feature, as they draw unproductive responses from the host.

My project aims to determine whether the central repeat or other PfCSP epitopes are immune-distracting and whether immuno-focused approaches can teach a host to evade the parasite's own evasive maneuvers. First, I will develop preclinical models expressing human PfCSP antibodies. Antibodies will either bind only a single PfCSP epitope (in/out of the repeat region), or will be dual-specific to the central repeat and at least one other PfCSP epitope. Second, I will investigate which epitopes impart a robust response. Humanized mice will be vaccinated with immunogens containing various segments of PfCSP. Third, I will generate mini-repertoires to mimic the host environment. B cells are in competition for antigen: to determine whether responses to the central repeat crowd out other responses by B cell competition, we will generate mice with multiple humanized B cell lineages, expressing precursors to antibodies to various parts of PfCSP.

I hypothesize that humoral immune responses to the central repeat will not be protective. Furthermore, immuno-focused responses excluding the central repeat will outperform broad responses. The Batista lab has developed an approach to rapidly generate mice with B cells bearing fully humanized B cell receptors (BCRs), as well as state-of-the-art immunocharacterization approaches to trace a single B cell lineage. This project advances those methods by generating models capable of expressing multiple PfCSP antibodies, recreating the competitive environment in humans in miniature.

Whether the central repeat performs an immune-evasive function will be determined by both direct measurement of the humoral responses (identifying which cells are activated, the amount and specificity of the circulating antibody response). I will also, and perhaps more tellingly, assess how vaccinated mice respond to a malaria challenge. This will provide either confirmation or refutation of this proposed mechanism by which parasites overcome acquired immunity not by avoiding a response, but by eliciting a harmless one.

Quantitative in vitro Approach to Virus Infection

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The study of phage biology and infection mechanisms is an important field of research that, since the first half of the 20th century, has delivered major scientific outputs for both fundamental scientific fields notably molecular biology and applied domains through applications like phage therapy. Viral entry process that goes from phage fixation to viral DNA ejection inside the host plays a major role in the phage biology governing central aspects, such as its infectivity and its specificity. It relies on the interaction between phage elements (notably phage tail and fibers) and bacterial ones, including protein receptors and elements from the cell wall. These mechanisms have been investigated for decades using various molecular and structural biology approaches. However, important knowledge gaps remain centered notably on quantitative aspects and non-model bacteriophages.

My project aims to unlock the potential of recent advances in the fields of biophysics and quantitative biology to achieve a deeper understanding of these mechanisms. The central goal is to reproduce the phage infection process in an in vitro setup by engineering synthetic cells encapsulating gene expression mixtures to be infected by natural or engineered bacteriophages. The infection of highly controlled synthetic cells by viral particles would be a game changer in the study of phage infection mechanisms. It allows for the quantitative assessment of the role of each partner involved and of important environmental conditions on the various mechanisms supporting the process of cellular phage entry. To achieve that goal, state-of-the-art methods will be applied to the engineering of both partners interacting, phage particles and synthetic cells. On the side of phage particles, their engineering and production will be performed on dedicated in vitro expression platforms using pipelines recently developed in-house that enable high throughput editing of phage candidates. In parallel, recent progress achieved on the engineering of custom lipid bilayers relying notably on the quartz crystal microbalance with dissipation monitoring (QCM-D) technology have enabled the tuning of synthetic cell membrane composition enabling the integration of various lipids and protein receptors.

We expect that this project will lead to major advances in our knowledge of phage biology. The possibility to systematically study the determinants of infection mechanisms should accelerate the development of mechanistic models describing more precisely these phenomena. In the long term, such an approach may also allow the acceleration of research on non-model phages as well as phages infecting hazardous or non-cultivable bacteria. Finally, in the long term, the details of this approach can also be transferred to eukaryotic cells to study the mechanisms governing their infection by viruses, opening new horizons for cell biology and virology.

Understanding the Host-microbe Arms Race Through the TLR5-flagellin Axis

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Hosts constantly encounter microbes at interfaces, such as the gut and respiratory mucosa. Pattern recognition receptors (PRRs) enable the host to rapidly detect conserved pathogen-associated molecular patterns and mount immune responses. Toll-like receptor 5 (TLR5) is a PRR which recognizes bacterial flagellin and drives inflammatory responses upon activation. TLR5 regulates the microbiome, and TLR5-deficient mice exhibit microbiome-dependent metabolic syndrome. Interestingly, TLR5 deficiency increases susceptibility to infection, but protects against inflammatory diseases such as Crohn's disease and Systemic lupus erythematosus.

Recent work has shown that bacteria have evolved different strategies during engagement of TLR5. Stimulator flagellins strongly bind and activate TLR5, thus, triggering an inflammatory response that benefits certain pathogens. Alternatively, pathogens may possess flagellins that do not bind and evade TLR5 signaling. Some commensals produce silent flagellins that bind TLR5 well, but do not activate TLR5, which contributes to immunological tolerance. PRRs may engage in co-evolutionary arms races with their microbial ligands at the host-microbe interface. The human TLR5 seems to be undergoing purifying selection. However, the functional TLR5F616L and TLR5N592S variants are maintained at relatively high frequencies. Although TLR5N592S is associated with ulcerative colitis and colorectal cancer and TLR5F616L is associated with Crohn's disease they may confer some benefits. These variants have only been characterized in the context of stimulatory flagellins.

We hypothesize that the F616L and N592S SNPs evolved to fine-tune TLR5 signaling in response to silent or stimulatory flagellins. These TLR5 variants may differ from the dominant allele with respect to their interactions with the two classes of flagellin. TLR5 heterodimers, which comprise monomers of the dominant TLR5 with TLR5 variant (1:1 ratio) may also exhibit altered binding and activation. We hope to achieve three aims with our work. First, examine the interaction of TLR5F616L and TLR5N592S with different flagellin types. Second, determine changes in TLR5 signaling after stimulation with different flagellin types. Third, investigate effects of TLR5 heterodimers on signaling.

We will examine the binding of representative silent and stimulatory flagellins to TLR5 variant monomers as well as homo and heterodimers. CRIPR-Cas9 based editing will be used to create homozygote and heterozygote knock-ins of TLR5 variants in human small intestine, colon and lung organoids. Subsequently, RNA-seq and ELISA will be used to determine responses to stimulation of organoids with representative flagellins. This study will improve our understanding of the role of TLR5 variants in disease and may yield new insights into PRR evolution. This work could also aid the development of therapeutics targeting TLR5 signaling.

Coupling Massive Gene Synthesis with Generative Sequence Models to Decipher Antibiotic Resistance

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The spread of bacteria expressing beta-lactamase enzymes, which hydrolyze beta-lactam antibiotics, threatens health systems worldwide. Despite the huge diversity of natural beta-lactamases, our knowledge of their sequence-function relationship focuses only on a handful of clinical isolates and is limited to single-mutational scans. I aim to characterize the whole sequence landscape of antibiotic-specific resistance by iteratively combining cutting-edge generative sequence models with high-throughput gene synthesis and functional assays. Protein language models are usually trained on unlabeled data. As a consequence, they are totally blind to function and only rely on statistical patterns to make predictions. To increase the accuracy of the models and endow them with biological meaning, I propose to develop functionally informed generative models by integrating in vivo measurements of thousands of diverged natural and artificially generated beta-lactamases.

The experiments will span all four beta-lactamase classes (A, B, C, and D) and use different bacterial species and beta-lactam antibiotics to reproduce clinically relevant conditions. To overcome the prohibitive costs needed to synthesize the libraries of long genes required by our project, I will work with the Tokuriki lab as they have considerable expertise with DropSynth, a cutting-edge multiplexed technique that uses microfluidic droplets to assemble oligo DNA sequences at very low prices. To generate artificial sequences for the first experimental round, I will employ a pre-trained autoregressive protein language model that will be initially fine-tuned on a curated dataset of beta-lactamase sequences extracted from public sequence databases. The function of natural and artificial enzymes will be quantified by linking it to bacterial fitness in a high-throughput fashion, which will be used to enrich the models with functional context. Comparing functional and inactive sequences will permit me to identify sequence patterns encoding functional specificity and go beyond the statistics of sequence alignments. Moreover, I will further develop the species- and substrate-specificity information by adding dedicated tokens at the beginning of the sequences and conditioning on them during the generative process in a fine-tuning step. The same procedure will be repeated in a second generative and experimental round to test the quality of the sequence landscape and refine the model once again.

The result will be a multidimensional, context-aware sequence landscape of antibiotic resistance for beta-lactamases. We anticipate that my work will shed light on beta-lactamase evolution and insights into the sequence space that encodes functional specificities will allow fast and inexpensive detection of novel, emerging, multidrug-resistant beta-lactamases.

Elucidating Electrophile-sensor Signaling Proteins at the Contact Sites Between Bacteria and Host

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The rise of antimicrobial resistance necessitates the development of new strategies and effective identification of actionable molecular targets/ligands. During invasion, bacteria release virulence factors, i.e., a myriad of proteins that aid in attachment to host (adhesins); they alter host signal transduction (toxins); and then they mimic host cell proteins (effectors). Infection begins by the interaction between adhesin proteins on the surface of bacterial cell wall and the corresponding receptors on the host cell surface, which results in adherence. The release of virulence factors during such contact elicits host immune response and results in oxidative stress and the release of membrane-permeable small-molecule metabolites, which are derived from polyunsaturated fatty acids (PUFAs). Peroxidation of PUFAs generates reactive lipid-derived electrophiles (LDEs) with antimicrobial functions. Recent data indicate that LDE-mediated host defense is counteracted by pathogen-specific proteins that render them viable therapeutic targets.

LDE signaling between bacteria and host cells during adherence is an overlooked treasure trove for novel small-molecule-targetable protein players. However, to date, accurate understanding of precision LDE-signaling mechanisms and associated context-specific players in host–bacteria interface is unavailable. A major bottleneck constitutes the lack of precision tools to perturb and probe these events contextually both in the systems- and protein/ligand-specific manners. Addressing such unmet capabilities will open the door to novel targets/ligands with new therapeutic modalities tapping into natural signaling mechanisms.

Our laboratory developed the REX (reactive electrophiles and oxidants) technologies, which enable interrogations into target-specific LDE signaling processes. Currently, REX-mediated precision localized electrophile delivery is the only means available to map locale-specific proteins for their context-specific spatiotemporal electrophile responsivity in live models.

Our project will adapt our REX technologies to achieve high-throughput mapping of actionable proteins of importance in bacterial–host adhesion, whose activity or function can be modulated by specific LDEs and related pharmacophores. Following functional validations and mode-of-action establishment, we will use the newly acquired mechanistic insights to uncover candidate ligand-target pairs that can mechanistically suppress bacterial adhesion.

Why Do Stressed Bats Shed More Viruses?

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Bats are reservoirs for many viruses, including coronaviruses (CoVs). Their unique physiology and immunity help them maintain these viruses without disease. Limited evidence suggests periods of active infections in bats (increased viral replication and shedding) are triggered by physiological stressors that provide opportunities for transmission to humans. Food scarcity and reproduction are two such stressors that correlate with viral shedding in bats.

However, the pathways through which they modulate viral replication and shedding are unknown. In this study, we will investigate the physiological manifestation of intrinsic and extrinsic stressors in fruit bats inhabiting intact and fragmented forests in Belize by integrating field studies, mathe-matical models, and in vitro experiments. Earlier studies have identified alphacoronaviruses (alpha-CoVs) in the bat *subfamily Stenodermatinae*, which includes genera *Artibeus, Dermanura, Sturnira*, and *Uroderma*. Using this host-pathogen system in Belize, we will test the role of physiological stress in viral pathogenesis.

We include three complementary aims with testable hypotheses. First, we will sample bats in wet and dry seasons to quantify physiological stress via neutrophil to lymphocyte ratios as well as fecal and hair cortisol. We will also standardize a qPCR assay to quantify heat shock proteins in blood. We hypothesize bats inhabiting fragmented forests will have elevated physiological stress. Stress will also vary seasonally and be highest in reproductive females. Generalized linear mixed models (GLMMs) will test the effects of season, habitat quality, and reproduction on stress. Next, we will detect alpha-CoVs in bat saliva and feces using RT-PCR and correlate it with stress biomarkers using GLMMs.

We hypothesize elevated physiological stress will increase viral replication and shedding, potentially mediated by immunosuppression. We will develop mathematical models capturing this relationship to test the sensitivity of various epidemiological parameters to physiological stress. Finally, we will develop cell line and organoid models for these bat species to validate our field observations and experimentally test our predictions. We hypothesize innate immune markers will have an inverse relationship with viral replication in cell lines and organoids and that both will respond to cortisol challenge in a dose-dependent manner.

We will challenge our models with an alpha-CoV with high similarity to CoVs found in Belize bats (HCoV-229E) in the presence of cortisol. We will use gene expression analyses to discover bat cellular response to stress in the face of CoV infection. For the first time, we will elucidate how stressors affect bat-borne zoonoses. Our work will represent a unique integration of field studies, mathematical models, and in vitro methods to allow mechanistic understanding and provide novel, cutting-edge approaches to test critically important ideas in the field of disease ecology.

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Synthetic Biology

Design and Build a Synthetic Single Cell Epigenetic Oscillator

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DNA packaging proteins called histones undergo covalent modifications causing epigenetic changes in gene expression. These changes are reversible leading to a switch between ON and OFF expression states without DNA sequence alterations. Such epigenetic switches must be robust during differentiation and yet susceptible to change in novel environments. We do not understand how epigenetic switches can be controlled to drive complex phenomena such as cellular differentiation and adaptation.

I propose to couple theory and experiments to build a synthetic epigenetic oscillator whose expression is tunable, reversible, and heritable with periodicities ranging from a few to many cell divisions. Developing this synthetic framework will reveal architectures that enable cells to maintain epigenetic memory that is robust and yet flexible to change. Histone H3 lysine 9 methylation (H3K9me) marks silent genomic regions called heterochromatin in fission yeast (Schizosaccharomyces pombe). Heterochromatin in S. pombe exhibits stochastic switching between ON/OFF expression states and serves as a versatile model system for my experimental goals.

Our hypothesis is that the H3K9me-dependent epigenetic switch relies on the activity and dosage of different chromatin regulators. By designing novel synthetic positive and negative chromatin-based feedback loops, we will transform a stochastic epigenetic switch into an oscillator. AIM 1: Develop a theoretical framework modeling epigenetic oscillations to identify the sources of noise that lead to stochastic switching between ON/OFF expression states. By modeling the interactions between chromatin modifiers and H3K9me, I will program the frequency of switching within cells and study their collective behaviors to determine how oscillations are coupled across cells. AIM 2: Implement theory-based predictions to build a synthetic epigenetic oscillator in S. pombe. Using microscopy and lineage tracing, I will visualize H3K9me dependent switching of a GFP reporter gene between ON/OFF states in individual S. pombe cells.

My work is a unique synthesis of theory and experiments made possible by working with Jane Kondev (Physics) and Kaushik Ragunathan (Biology) at Brandeis University. The Kondev lab develops mathematical models centered on chromosome organization and gene regulation while the Ragunathan lab has expertise in using genetic and biochemical tools to inducibly control H3K9me epigenetic switching in S. pombe. Using precision engineered genetic tools, I will implement novel H3K9me dependent positive and negative feedback loops to produce oscillations in expression that will be further refined by additional theory. Building a tunable single cell epigenetic oscillator will break new ground in our understanding of epigenetic switches and would eventually lead to experiments in tissues where synthetic oscillators can program responses to an external stimulus in a coherent manner.

Teaching Excitons How to Walk: Spatial Reorganization in an Artificial Light-harvesting System

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Supervised by Gabriela Schlau-Cohen

Photosynthetic organisms rely on light to synthesize important chemical feedstocks. On a molecular level, the flow of protons and electrons, after absorption of a photon, is controlled by sophisticated nano-machinery. Through this complex dance, biological systems power most life on Earth. Inspired by this remarkable achievement, researchers have been working to emulate this function through artificial model systems. Despite decades of effort, robust and efficient energy capture has not been achieved. Fundamentally, biological systems are non-equilibrium in nature. However, previous reports have been limited to static, equilibrium geometries, and so structural dynamics that are inherent to biological function have remained unexplored.

While there is vast knowledge on how nature uses light to fuel life, true understanding is proven by successful replication of the process. In the words of Richard Feynmann, «What I cannot create, I do not understand.» This project aims to create synthetic nano-machinery with dynamic control over the excitons for energy capture and conversion. This platform will establish the limits of our current understanding and provide fundamental insight on the interplay of biological structure and dynamics.

I propose to construct a system at the molecular level with dynamic control over photonic energy. Specifically, I will program excitonic circuits made of dyes and charge-transporting moieties with tailored non-covalent interactions that introduce dynamic couplings. After initial conversion of photonic energy to an electron-hole pair, these transient bonds break, which pushes the system out of equilibrium. The resulting energy gradient triggers molecular reorganization and induces a spatial separation of charges, which can be harnessed to drive chemical transformations.

Precise control over the system's geometry is necessary to exploit their full light-conversion potential. Synthetically, this can be achieved with a powerful biotechnological tool known as DNA origami. The efficiency of the system is determined by elementary steps such as energy transfer and charge separation. As these processes occur on ultrafast timescales, time-resolved optical spectroscopy with femtosecond resolution will be used to track the different species via their distinct absorption features. These in-depth spectroscopic studies will be used to fine-tune the molecular design of the artificial light-harvesting systems.

This project expands our understanding of photosynthesis by reverse-engineering its function in minimalistic excitonic circuits. The combination of atomically precise molecular design and state-of-the-art time-resolved spectroscopy allows identifying the key excitonic processes and optimizing them for maximum efficiency. Overall, this project will provide invaluable insight on one of the overarching questions in biology: how structure creates function.

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By nationality of scientist and host institution

Dual nationalities noted with*

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