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ORAL SESSIONS

ARTIFICIAL ALLOSTERIC PROTEIN SWITCHES WITH MACHINE LEARNING-DESIGNED RECEPTORS

Kirill Alexandrov
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Protein allostery underlies most information and energy processing in biology, and the development of artificial allosteric proteins is a key objective of synthetic biology and biotechnology. We demonstrate that minimalistic ligand-binding domains designed using machine learning algorithms serve as efficient synthetic receptors in one-component allosteric protein switches. These include highly effective colorimetric, luminescent, and electrochemical biosensors for small molecules, peptides, and proteins, which can be complied into intramolecular YES and AND logic gates. Furthermore, we report fully synthetic allosteric switches composed of artificial receptors and reporters. We conclude that allostery emerges spontaneously in artificial proteins and enables the construction of protein switches with customizable inputs and outputs. The practical utility of this approach is demonstrated by engineering E. coli cells with ligand-dependent antibiotic resistance and developing bioelectronic devices capable of quantifying steroid hormones. Our work lays the foundation for the development of complex bio-circuitry for real-time information processing.

Keywords: Protein Design, Protein Allostery, Protein Biosensors, Artificial Proteins, Protein Switches

HFSP Reference Number: RGP0002/2018
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2018

CHIKUNGUNYA VIRUS-MEDIATED MODULATION OF INSECT CELL MEMBRANE COMPOSITION AND FUNCTION.

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Insect cell membranes are highly elastic compared to mammalian cell membranes, but the underlying mechanism of these differences in properties is poorly understood. To dissect how such a fundamental difference in deformability exists, we analysed the composition of the insect cell plasma membrane using a novel toolkit coupled with advanced microscopy methods. To explore these properties, we have utilised chikungunya virus (CHIKV), an (+) single stranded RNA alphavirus. CHIKV is an arbovirus that generates a significant disease burden in humans and replicates through a mosquito host, aedes albopictus. During its cellular replication cycle, CHIKV generates a membrane microdomain for RNA replication termed a spherule. Here, we utilise this membrane microdomain to understand the compositional differences between mammalian cells and insect cells. Using a library of lipid probes we characterise these domains as enriched in a specific lipid profile that is distinct from the general plasma membrane. We perform high-resolution structural analyses using electron microscopy to show fundamental differences in the replication potential of CHIKV between mammalian cells and insect cells. We have also developed assays to screen a library of CRISPR edited lines for to determine genes that are involve in controlling viral entry, replication, and egress. In conclusion, our study elucidates the fundamental differences in membrane properties between mammalian and insect cells, shedding light on how these differences influence the replication of CHIKV. By revealing the molecular and structural differences in the spherule microdomains, we hope to uncover potential targets for therapeutic interventions that could disrupt viral replication by modulating membrane properties. Furthermore, the use of CRISPR-edited cell lines in our screening assays will allow for a more targeted investigation of specific host factors involved in the lifecycle of CHIKV, potentially identifying key host proteins or lipids that could serve as novel drug targets. Eventually, our findings will enhance the understanding of CHIKV biology and offer broader insights into how membrane dynamics influence virus-host interactions across other similar viral pathogens.

Keywords: Membrane Biology; Chikungunya Virus; Insect Cells

HFSP Reference Number: RGP011/2023
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

HOW LIFE GOT MOVING: RECONSTRUCTING AND RE-EVOLVING THE BACTERIAL FLAGELLAR MOTOR

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The ability to move is a fundamental trait of life, yet the origin and evolution of motility remain open questions. We investigate this by focusing on one of the oldest motility systems: the bacterial flagellar motor (BFM). The BFM is a complex, ion-powered rotary motor that drives rotation of the flagellum – and dates back to the last common bacterial ancestor. Over the last three years of HFSP-funded research, we have integrated phylogenetics, protein structure modeling, ancestral sequence reconstruction, and experimental evolution to characterize the origins and adaptations of flagellar motility. We examined how bacterial flagellar stators adapt to environmental challenges (DOI: 10.1126/sciadv.abq2492). Using CRISPR engineering, we replaced the native H+-powered stator in Escherichia coli with a Na+-powered variant and observed its rapid evolutionary reversion under low-sodium conditions. Whole-genome and RNA sequencing revealed key genetic adaptations, demonstrating the flexibility and hierarchical modularity of the stator complex. These findings highlight how molecular machines adapt in real-time, providing a direct link between environmental pressures and the evolution of motility components. To further investigate flagellar function, we performed the first phylogenetic and structural classification of the flagellar stator complex (DOI: 10.1101/2024.07.22.604496). We identified two major stator classes: the Flagellar Ion Transporters (FIT) and the more diverse Generic Ion Transporters (GIT). FIT proteins possess a torque-generating interface (TGI) crucial for motor function. Functional assays in Escherichia coli confirmed that these structural elements are essential for flagellar rotation. Building on these insights, we engineered chimeric ion-powered rotary motors (DOI: 10.1128/jb.00140-24). Through directed evolution, we identified adaptive mutations that enhanced motility. Our analysis provides a foundation for understanding the diversification of stator proteins and their common origin. More broadly, our work contributes to wider efforts to integrate phylogenetics and experimental evolution to examine early flagellar history.

Keywords: Flagellar, Motility, Bacteria, Molecular Motor, Evolution

HFSP Reference Number: RGY0072/2021
HFSP Award Category: Alumni Young Investigator Grant
HFSP Award Year: 2021

HIGH-THROUGHPUT SYNTHESIS AND EVALUATION OF ANTIVIRAL COPOLYMERS FOR ENVELOPED RESPIRATORY VIRUSES

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ABSTRACT
COVID-19 made apparent the devastating impact viral pandemics have on global health and order. Development of broad-spectrum antivirals to provide early protection upon the inevitable emergence of new viral pandemics is critical. The Medicinal Molecules and Materials Group at QUT (https://research.qut.edu.au/medicinal-molecules-materials/) are discovering new antiviral polymers using a combination of high-throughput polymer synthesis and antiviral screening, supported by AI, enabling diverse polymer compositions to be explored rapidly.

Our high-throughput approach has identified that amphipathic polymers, with ionizable tertiary amine groups are the most potent antivirals, with minimal cytotoxicity. They have demonstrated broad spectrum efficacy against a range or respiratory viruses including influenza virus and SARS-CoV-2. It is hypothesized that these polymers interact with the viral membrane as they showed no activity against a non-enveloped virus (Rhinovirus). This presentation will showcase how the polymers were discovered, and the switchable chemistry of the polymers during endosomal acidification was evaluated using lipid monolayers, indicating a complex synergy between hydrophobicity and ionization drives polymer-membrane interactions. Preliminary preclinical evaluation of the lead candidates is currently ongoing, and results from model endothelial experiments with primary patient cell lines, and in vivo models, will showcase the promise of this novel class of antiviral materials.

Keywords: Polymer, Antiviral, Virus, Nanomedicine

HFSP Special Invitee
Masterclass Australia 2025

RECEPTOR-NORMALIZED GENERALIST-SPECIALIST VIRAL CO-INFECTIONS IN MARINE VIBRIO BACTERIA

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The antibiotic resistance crisis led to the resurgence of bacteriophage therapy, where dozens of different bacterial-specific viruses (phages) are simultaneously administered to treat human pathogens (phage cocktails). Although phage cocktails can lead to different phages co-infecting the same bacterium, what governs the outcomes of co-infections in wild bacteria is unclear. The problem behind the total absence of phage co-infection studies is that phages can only infect very specific strains, due to receptor type and bacterial anti-phage defenses, and it is thus rare to obtain bacterial hosts co-infectable by multiple different phages. To solve this problem, we used the Nahant collection [1], a matrix of >2,000 wild marine Vibrio strains, that were individually used to isolate >250 sympatric phages. We obtained many dozens of co-infectable bacterial hosts by focusing on phage 1.215.A., a generalist that can infect >100 Vibrio strains, but with a large fitness spectrum across different hosts, many of which are suboptimal for phage replication. The same strains are often infectable by a second, specialist phage. Importantly, by genetically transferring the optimal receptor of 1.215.A. from one strain to all others, we show that receptor incompatibility reduces phage 1.215.A. fitness in around half of the suboptimal strains, whereas in the other half, phage fitness is reduced due to intracellular bacterial anti-phage defenses. Thus, we precisely assess how often defenses affect phage proliferation in an ecological setting, and test whether generalist-specialist phage co-infection outcomes change upon host defense activation.

1. Kauffman, K. M. et al. Resolving the structure of phage–bacteria interactions in the context of natural diversity. Nat. Commun. 13, 372 (2022).

HFSP Reference Number: LT0033/2023-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

Keywords: Bacteria, Viruses, Bacteriophages, Co-infection, Genetics

FROM EXPLORING THE CONCEPT OF ADAPTIVE IMMUNITY TO VIRUSES IN MOSQUITOES TO SIGNITURES OF SELF-DOMESTICATION

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The mosquito Aedes aegypti is the primary vector of arboviruses such as dengue, zika and Chikungunya viruses, which are a risk for about half of the world's population. Aedes aegypti is also an invasive species, whose movement out of its native range in Africa was accompanied with its differentiation into two ecotypes: Ae. aegypti formosus (Aaf) remained in Africa, while Ae. aegypti aegypti (Aaa) distributed globally. Differently than Aaf, Aaa evolved to specialize in biting humans for blood-feeding and oviposite in clean water of artificial containers, making it a more efficient vector than Aaf. A deep-rooted hypothesis among vector biologists is that Aaa emerged through self-domestication, a process whereby a species evolves in response to conspecific-exerted selection pressures that mimic domestication, but without humans serving as a domesticator. When sequenced, the genome of Ae. aegypti revelaved expansions in gene families associated with chemosensation, detoxification and immunity and richness (>50%) of repetitive sequences, including the presence of hundreds of fragmented viral sequences, which we named non-retroviral endogenous viral elements (nrEVEs). We showed that nrEVEs are enriched in piRNA clusters, regions of the genome, which contain sequences of previously-acquired transposable elements that they control trough small RNA sequences called piRNAs. Consequently, we hypothesised that nrEVEs constitute memories of previous viral infections and contribute to contain subsequent cognate infections.
Through the collection, sequencing and analyses of the genomes of > 600 Ae. aegypti mosquitoes, including Aaf and Aaa samples, we showed that integration of viral sequences is a rare and continuous event, but does not correlate with the intensity of viral exposure nor the shift between Aaf and Aaa. Instead, we found evidence of adaptations from self-domestication in genes associated with broad chemosensory, neuronal, hormonal and metabolic functions, highlighting striking similarities with functions of adaptive genes associated with human-driven domestication of not only other insects as the silkworms, but also chickens, rabbits and cattle. We also showed that self-domestication processes have occurred, and many continue to occur, in Ae. aegypti, because of extensive standing variation in the genome, which allows local adaptations, and the neuronal-olfactory redundancy, with many neurons co-expressing multiple receptors with different chemical sensitivities. Our results highlight a link between the evolution of the species and its genome complexity, which should be further investigated.

Keywords: Mosquitoes, Immunity, Viruses, Invasion, Domestication

HFSP Reference Number: RGP0007/2017
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2017

PROBING THE EVOLUTIONARY ECOLOGY OF COGNITION THROUGH HIGH-DENSITY DIFFUSE OPTICAL TOMOGRAPHY

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Most of our knowledge on the inner workings of the vertebrate brain relies on generalizations from a very small number of species or from comparisons of easily attainable metrics that, like brain size, offer very few insights into the mechanisms involved. Broadening the taxonomic scope of these analyses has proven difficult due to a lack of portable, non-invasive, and cost-effective technologies for sampling brain anatomy and function within reasonable timeframes. Our global team of radiologists, avian neuroscientists, and evolutionary ecologists has secured HFSP support to address these challenges through the development and implementation of optical imaging technologies that allow us to map the functional connectivity patterns of the avian brain. In this talk, we will share exciting new brain imaging results captured through Optical Intrinsic Signaling (OIS) and Diffuse Optical Tomography (DOT) on pigeons. Our findings demonstrate that optical imaging is a viable alternative to fMRI in birds, yielding accurate characterizations of brain functional connectivity networks of similar quality and resolution but at a fraction of the cost, in field or laboratory settings, and without the need for sedation.

Keywords: Optical Brain Imaging, Functional Connectivity, Evolutionary Ecology Of The Vertebrate Brain
HFSP Reference Number: RGP003/2024
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2024

TUMBLEWEED: AN ARTIFICIAL MOTOR PROTEIN THAT WALKS ALONG A DNA TRACK

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Life is characterized by directed motion on all length scales. At the subcellular level, Nature has evolved several molecular machines that produce directed motion, usually powered by free energy gained from ATP hydrolysis. In most cases, Nature has used proteins as the material of choice from which to fashion molecular motors. The sophisticated and nuanced functions of protein motors are afforded by the chemical and structural complexity of proteins, which are key to their success in evolution. From a synthetic biology perspective, there have been spectacular advances in the creation of artificial molecular motors. Although small-molecule- and DNA-based molecular motors have been synthesized, the creation of an artificial motor protein remains a significant challenge in synthetic biology. To tackle this, we have taken a modular approach, where we take well-characterised functional components from natural non-motor proteins and assemble these in new ways so that motor function emerges from non-motor protein domains. Using this strategy, we have created the Tumbleweed (TW), an artificial protein walker capable of directional movement along a DNA track, powered by changes in chemical potential supplied via microfluidics. TW is a three-legged clocked walker composed of three α helical coiled-coil 'legs' connected via a SpyTag/SpyCatcher hub. Each leg terminates in a distinct ligand-gated DNA-binding domain, a 'foot', that controls binding of TW to a specific site on a DNA track. The DNA track is composed of an ordered array of binding sites, each the target for one of the three TW DNA-binding domains. By dynamically modulating the concentrations of the three controlling ligands, we can control both binding and stepping of TW along the track. TW works via a Brownian ratchet mechanism where steps are effected by diffusion and then rectified by the controlling ligands. Using single-molecule fluorescence assays, we show that TW steps directionally along a DNA track when the ligand concentrations are altered in a prescribed sequence. Currently, TW takes a 17 nm step every 7 seconds, where the timing is dictated by our microfluidic device. We observe up to 11 consecutive steps before either TW detaches from the track or its fluorescent label photobleaches. Our challenge now is to improve the performance of TW and use it as a stepping stone towards our ultimate goal of creating an autonomous artificial protein motor.

Keywords: Synthetic Biology, Molecular Motors, Protein Design, Motor Protein, Single Molecule

HFSP Reference Number: RGP0031/2007
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2007

BUSTING MILES: OPTOGENETIC TAMPERING OF THE FLY ODOMETER

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Estimating travel distance is fundamental to path integration in a wide range of animals, including insects, birds, and vertebrates. Despite its importance, the mechanisms by which velocity-sensitive neuronal outputs are integrated to estimate distance remain poorly understood. Current insect model systems for studying path integration face significant challenges: They often rely on species with limited genetic accessibility or require complex transgene combinations, making them unsuitable for large-scale genetic screens. To address these challenges, we developed a novel memory-based assay that trains flies to associate specific travel distances with aversive electric shocks. Flies trained in thiso paradigm exhibited avoidance behaviors, spending less time in shock-associated areas and reversing direction before reaching the shock-associated distance, with memory persisting for up to an hour. We found that olfaction is essential for place avoidance but not for estimating travel distances. To better understand the process, we constructed an agent-based model in which an odometer-like system integrates translational velocities during walking. This model was then used to predict how naive flies, flies undergoing associative conditioning, and trained flies would adjust their travel distances when values were synthetically added to the integrator. Through a genetic screen, we identified neurons in the central complex of the fly brain that are integral to odometry. Our hits overlapped with the circuit that encodes translational velocity in Drosophila. To test our model's predictions, we applied brief optogenetic stimulations to these neurons during different stages of the task. The behavioral outcomes aligned closely with the model's predictions. Our findings provide compelling evidence that translational velocity integration underpins distance estimation in insects and shed light on the neural circuitry responsible for this critical function.

Keywords: Navigation, Path Integration, Neural Circuit, Drosophila

HFSP Reference Number: CDA00011/2016-C
HFSP Award Category: Alumni Career Development Award
HFSP Award Year: 2016

MAPPING SOCIAL PERCEPTION TO SOCIAL BEHAVIOR USING ARTIFICIAL NEURAL NETWORKS

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Primates possess sophisticated social cognitive abilities, interpreting complex social cues and producing adaptive behaviors in real-time. These abilities are thought to rely on distributed circuits spanning sensory, limbic, and prefrontal brain regions. Despite some progress in identifying neural correlates of specific social features, such as gaze and social rank, a comprehensive computational framework linking social perception to behavior remains elusive; at least in part due to the high dimensionality of both the input space (complex social scenes) and the output space (diverse behavioral responses).
To address this, we combine artificial neural networks (ANNs) and high-density Neuropixels electrophysiological recordings to uncover how the primate brain transforms dynamic social scene perception into social behavior. We first employ whole-brain functional magnetic resonance imaging (fMRI) to locate brain regions selective for social video in macaques, specifically identifying the dorsal bank of the superior temporal sulcus (dSTS), orbitofrontal cortex (OFC), and anterior insula (aIC). We then target these regions using Neuropixels probes, revealing strong selectivity for social stimuli on a single-unit level. By training a set of neural networks to embed and reconstruct videos from neural activity, we surprisingly observe that neural responses in OFC and aIC support astonishingly accurate reconstructions of social videos. We further extend this approach to generate optimal stimuli for neurons from each region and identify interpretable axes of neural responses like social partner angle and distance. Finally, we identify a subset of neurons predicting social behavioral responses and observe a causal role of activity in aIC and OFC in social behavior production, with electrical microstimulation evoking gaze shifts and facial movements.
These results suggest that these frontal brain regions contain a surprisingly rich code for social scenes and play a critical role in transforming social perception into behavior, positioning them as central nodes in social cognition.
Keywords: Neuroscience, Vision, Behavior, Social cognition, Machine learning

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HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2022

STABILITY PROTEOMICS – A TRANSFORMATIVE EXPERIMENTAL TOOLSET FOR IDENTIFICATION OF PROTEIN-LIGAND INTERACTIONS

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Identifying drug targets for novel small-molecule therapeutics discovered through phenotypic screening remains a major challenge in antimalarial drug development. Stability Proteomics methodologies—untargeted and unbiased approaches for identifying drug-binding proteins in an organism—offer a powerful solution to this knowledge gap. These methods exploit the fundamental principle that ligand binding stabilizes proteins. By leveraging this concept, several orthogonal techniques have been introduced, which interrogate the proteome and detect drug-induced change in stability based on target protein's altered susceptibility to denaturation by temperature, organic solvents, or proteolytic cleavage. To advance antimalarial drug discovery, we developed next-generation experimental and analytical workflows for three complementary and orthogonal Mass Spectrometry based Stability Proteomics approaches: Thermal Proteome Integral Solubility Alteration Assay (Thermal PISA), Solvent PISA, and Limited Proteolysis Mass Spectrometry (Lip-MS). We first applied these methods to characterize target engagement and downstream mechanism-of-action (MoA) effectors for a diverse library of antimalarial compounds, including clinically approved drugs, tool compounds, and leading candidates in clinical trials. In parallel, we leveraged these new methods to interrogate core biological processes in parasite development, that to this date remain poorly understood. Deploying three-dimensional experimental approach, we gained novel insights into the sequence of events governing activation of sexual parasite stages gametocytes. This work provides a new layer of information, complementing existing datasets and enabling rational design of the next generation antimalarial therapies with transmission blocking activity. Overall, our findings demonstrate the universality, sensitivity, and specificity of these methodologies in identifying protein-ligand interactions, and highlighting Stability Proteomics as a transformative approach for accelerating target deconvolution in drug discovery, and characterising biological processes, with potential applications extending well beyond our model organism Plasmodium falciparum.

Keywords: Malaria, Drug-Target Identification, Proteomics, Parasite Transmission

HFSP Reference Number: LT-001/2022
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2022

CHARTING THE LIPIDOMIC COMPLEXITY OF THE SYNAPSE

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The brain is an extremely lipid rich organ with a complex and cell/organelle-specific composition. Lipids have critical functions in neuronal processes such as membrane formation and signal transmission, while diversity in lipid composition is associated with evolution of higher order cognitive abilities and instrumental for synaptic plasticity. The synaptic junction is the central building block of a chemical synapse that mediates cell–cell contact and signal transduction. Compelling evidence suggests a crucial role of lipids in synaptic neurotransmission and it remains unclear whether the molecular diversity of lipids impacts circuit-specific differences in neurotransmission and differential predisposition to synaptic dysfunction. Novel technologies, including mass spectrometry imaging and chemical tagging, now exist to study lipid function with the necessary molecular and spatial detail to resolve the lipidome of synaptic junctions. First, we developed a comprehensive and highly confident lipidomic workflow to chart the lipid profiles of different types of synapses from neurons of the hippocampus, cerebellum and striatum covering > 1000 lipids from 42 lipid classes. Cholesterol and glycerophospholipids are the most abundant (27 lipid species account for 75% of the lipidome) and glycosphingolipids (i.e. HexCer, Hex2Cer, SHexCer, GD1 and GM3) the most significantly regulated lipid classes in the synaptic junctions of the various brain regions. We have also expanded the structural detail by which lipids are identified in the synapse. Using novel isomer-resolved MS methods we have revealed the profile of isomeric lipids (double bond an sn-isomer level) within the synapse) for the first time. Applying this analysis to synapse isolated from mice in the presence of and without an enriched environment we have found that lipids containing certain polyunsaturated fatty acids have altered isomer populations, possibly result of membrane acyl chain remodeling. This points to the possibility that lipid modifications might be related to improved cognitive function in mice. Moving beyond pooled lipid extracts we havesought to develop and apply new technologies to map lipid composition throughout brain regions at cellular and sub-cellular resolution. We have developed a mode mass spectrometry imaging (MSI) system enabling us to map lipid complexity throughout neuronal tissues at up to 1 micron spatial resolution, revealing the complex spatial arrangement of lipids. As steps towards single organelle lipidomics we have are investigating this technology to map lipid composition and diversity amongst individual synapse collected from different brain regions. The developed LipidSpace platform allowed the in-depth comparison of the various lipidomes, giving insights on the biological function and diversity of synapses. Initial results from this ambitious work will be presented. Our ultimate goal is to use our technological advances to investigate the synaptic lipidome of different neuronal subtypes and synapse diversity within different brain regions.

Keywords: Synapse, Lipidomics, Mass Spectrometry, Single Cell, Chemical Tagging
HFSP Reference Number: RGP0002/2022-101
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

MOLECULAR DEFINITION OF THE ENDOGENOUS TOLL-LIKE RECEPTOR SIGNALING PATHWAY

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Toll-like Receptors (TLRs) are key mediators of the immune response to infection. Upon detection of microbes, these prototypical pattern recognition receptors activate inflammatory signal transduction pathways that involve IκB kinases (IKKs), mitogen activated protein kinases (MAPKs), ubiquitin ligases (e.g. TRAF6) and other adaptor and signaling proteins. Current models suggest these signaling proteins operate within functionally distinct multiprotein complexes, which are activated by a receptor-linked adaptor complex known as the myddosome. The mechanisms that connect the protein complexes in the TLR pathways are undefined. To delineate TLR pathway activities, we genetically engineered human and mouse macrophages to add a novel epitope tag to the endogenous myddosome constituent MyD88 which enabled live cell imaging and proteomic analysis. We found that MyD88 forms transient contacts with activated TLRs and that myddosomes dissociate from their seeding TLRs. Cytosolic, TLR-free myddosomes are dynamic in size, number, and composition. Microscopy revealed that MyD88 forms a scaffold for effector protein recruitment, proteomics demonstrated that myddosomes contain proteins that act at all stages and regulate all effector responses of the TLR signaling pathways, and genetics defined the epistatic relationship between these effector modules. Based on these findings, we propose that the entire TLR signaling pathway is executed from within the myddosome.

Keywords: Toll-like Receptors, TLR, MyD88, Myddosome

HFSP Reference Number: LT0006/2022-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2022

CAPTURING LONG-TERM HIPPOCAMPAL DEVELOPMENT IN VITRO USING A NOVEL STEM CELL-DERIVED 3D BRAIN ORGANOID MODEL SYSTEM

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Murdoch Children's Research Institute

The hippocampus is a brain structure that plays an essential role in memory and learning. Impairments in these cognitive functions are associated with neurodevelopmental disorders, such as intellectual disability and epilepsy. However, the cellular and molecular processes underlying these functions remain largely unknown. Stem cell-derived 3D organoids provide an invaluable opportunity to expand our understanding of the human hippocampus's complex cellular and functional networks in health and disease. By exposing iPSC aggregates to BMP-WNT signalling and adapting the cultures to growth in spinner-flask bioreactors, we have established a new 3D organoid model for long-term hippocampal development in vitro. Single-cell RNA-sequencing of individual organoids cultured at 1, 3, and 6 months showed high organoid-to-organoid reproducibility and a stronger correlation with the foetal hippocampal tissue compared to other human brain regions between 12 and 21 PCW. The gene expression profile of cell types observed in organoids also reveals a region-specific and time-dependent appearance that aligns with those found in developing human hippocampal tissue. Indeed, a significant correlation for radial glia, as well as both excitatory and inhibitory progenitor cells is shown during the early differentiation stage. Conversely, interneurons, astrocytes, oligodendrocytes, pyramidal cells in the Cornu Ammonis, and PROX1+ cells of the dentate gyrus are observed in the later times, demonstrating that the new model resembles the cell composition and developmental time course of human brain development. We also assessed the neuronal connectivity and network dynamics during hippocampal organoid differentiation in 3D space, leveraging a non-invasive and high-throughput microelectrode array. Our findings reveal that synchronised burst firings and local field potentials begin during the early differentiation stage. As time progresses and further cellular complexity is reached, a more intricate neuronal network emerges, recapitulating the functional timeline of embryonic brain development. This new 3D model provides an unprecedented platform to investigate how the hippocampus develops and identify the pathophysiological mechanisms of neurological disorders affecting this brain region.

Keywords: Stem Cell-Derived 3D Brain Organoids, Hippocampus, Disease Modeling, Neurological Disorders

HFSP Reference Number: LT 0024/2022-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2022

EXPLORING THE MICROBIAL DARK MATTER USING ELECTRON CRYOTOMOGRAPHY

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DPANN archaea account for half of all archaeal diversity in the biosphere and are characterized by their small size, reduced genome, and limited metabolic capabilities. Consequently, most members of the DPANN superphylum live in a mutualistic, commensal, or parasitic relationship with diverse archaeal and bacterial hosts. Despite their widespread occurrence and significant role in microbial ecology and the environment, very little is known about their cell biology, metabolic potential, and the molecular/structural basis of their host interactions. A significant hurdle in the study of DPANNs lies in the formidable difficulty of isolating and establishing new co-culture systems.
We used cryo-electron tomography (cryoET) to image several different host-DPANN co-cultures. Tomographic reconstructions combined with 3D segmentation uncovered two novel molecular machines that facilitate intercellular interaction between the host and DPANN. In one co-culture, we show that host cells can make extensive protein nanotubes to interact with their DPANNs. In another host-DPANN system, we observed DPANN cells assemble an enormous attachment organelle that makes intricate connections with the host, bridging the two cytoplasms. Our work shows the vast resources that both host and DPANN commit to this symbiosis and provides mechanistic insights into the DPANN-host relationship.

Keywords: Microbial dark matter, DPANN archaea, Electron cryo-tomography, in situ structural biology

HFSP Reference Number: RGEC33/2023
HFSP Award Category: Alumni Research Grant Early Career
HFSP Award Year: 2023

DISSECTING VIRAL PROTEIN MULTIFUNCTIONALITY – A PATH TO UNDERSTANDING HOW DEADLY RNA VIRUSES REMODEL THE HOST CELL

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Despite having extremely small genomes, RNA viruses include some of the deadliest human pathogens, such as Nipah virus, Ebola virus and SARS-COV-2. Many RNA viruses encode for less than ten proteins, a tiny fraction of the approximately 20,000 proteins encoded by humans, and yet are capable of commandeering and disabling host cell biology to promote virus propagation, transmission and disease. Proteins from RNA viruses therefore need to be highly multifunctional, but how such multifunctionality is coordinated is poorly understood. Like all proteins, viral proteins function largely through protein-protein interactions and participation in multiprotein complexes. Proteins from RNA viruses often form extensive interactions with viral and host proteins. Importantly, some viral proteins also traffic between subcellular compartments, including the cytoplasm and nucleus, to partition their many functions/interactions. This is particularly interesting for RNA viruses that replicate exclusively in the cytoplasm, as trafficking of proteins to the nucleus is indicative of 'accessory' functions, such as suppressing the host immune response. However, for many viruses/proteins, nuclear functions remain unresolved. Here, using extensive quantitative cell imaging, protein-protein interaction analyses and molecular mapping, we have identified multiple interactions of viral proteins with the host nuclear transport machinery, including VP24 protein from the highly pathogenic Ebola virus, and nucleocapsid from SARS-COV-2, indicative of novel nuclear functions. Next, applying advanced proteomic approaches, including high-throughput mass spectrometry and protein correlation profiling, to study the matrix protein from the deadly Nipah virus, we have defined the virus:nuclear interface in unprecedented detail. We identify and describe the complement of discrete protein complexes formed and altered by matrix protein, including complexes involved in fundamental host cell processes such as pre-mRNA splicing, ribosome biogenesis, translation and protein turnover. Importantly, subsequent validation of selected complexes has revealed viral modulation of host intranuclear bodies and RNA metabolism pathways. Together, the data provide unparalleled insight into how multifunctional proteins from highly pathogenic RNA viruses hijack and remodel the host cell, which will be of significant value for the development of antivirals and live-attenuated vaccines.

Keywords: RNA Virus, Protein, Virus-Host Interactions, Infectious Disease

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HFSP Award Category: Alumni Long-Term-Fellowship
HFSP Award Year: 2021

BACTERIAL TARGETING OF THE HOST EPITRANSCRIPTOME

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Post-transcriptional processing and modification of messenger RNA (mRNA) regulates gene expression in eukaryotes by dictating the stability, localisation and translation of newly synthesised transcripts. These epitranscriptomic events are coordinated by an extensive network of RNA-binding proteins. Here we observed that the bacterial pathogen, Legionella pneumophila, caused the selective degradation of host cellular mRNAs encoding factors involved in glycolysis and related metabolic pathways. Screening of a library of L. pneumophila deletion mutant strains revealed a single effector protein, LegC4, that mediated the post-transcriptional degradation of host mRNAs encoding key glycolytic enzymes, thereby suppressing host glycolysis during infection. Using CLIP-seq and complementary methods, we observed that LegC4 bound to mature processed host mRNA recognising a guanine (G)-rich motif that was overrepresented within mRNAs targeted for degradation. In vitro activity assays showed that LegC4 harboured intrinsic RNase activity and structural determination of a catalytically inactive mutant of LegC4 in complex with single-stranded RNA revealed a unique RNA-binding domain. The selective binding and degradation of host mRNA by LegC4 reveals a previously undescribed mechanism of bacterial effector protein activity targeting the host epitranscriptome.

Keywords: Host-Pathogen Interaction, Epitranscriptome, RNA Binding Proteins, Bacterial Infect
Receptor-normalized generalist-specialist viral co-infections in marine Vibrio bacteria
ion

HFSP Reference Number: RGP013/2023
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

VARIANT RIBOSOMAL DNA IS ESSENTIAL FOR FEMALE DIFFERENTIATION IN ZEBRAFISH

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The ribosome consists of protein and RNA components. Deletion of genes encoding specific ribosomal proteins has revealed that heterogeneity in the ribosome must exist in vertebrates; however, this has not been tested for ribosomal RNA (rRNA). In zebrafish (Danio rerio), the '45S-M' ribosomal RNA-encoding locus undergoes massive extrachromosomal amplification during oocyte growth and ovary differentiation and is distinct from the regular ribosomal DNA (rDNA) locus encoding somatic rRNA (45S-S). Although the 45S-M rDNA locus falls within the only described sex-linked region in multiple wild zebrafish strains, its role in sexual differentiation is unclear. We used CRISPR–Cas9 gene editing to alter 45S-M rDNA sequences in zygotes and found that although there was no effect on growth or male development, there was dramatic suppression of female differentiation. Males with edited 45S-M rDNA produced phenotypically normal sperm and were able to fertilize eggs from wild-type females, with resulting embryos once more displaying normal development. Our work supports the hypothesis that specialized 45S-M rDNA is the elusive apical sex-determining locus in zebrafish and that this region represents the most tractable genetic system to date for studying ribosomal RNA heterogeneity and function in a vertebrate.

Keywords: Ribosome Heterogeneity, Genetics, Sex Determination

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HFSP Award Category: Alumni Long-Term-Fellowship
HFSP Award Year: 2009

EXOME CAPTURE OF MITOCHONDRIAL SYMBIONT MIDICHLORIA IN TICKS DURING BLOOD-FEEDING PROVIDES NEW INSIGHTS INTO A UNIQUE ENDOBIOSIS

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Ixodes ricinus are obligate hematophagous ticks (Ixodidae, Arthopoda) whose single blood meal drives ovary maturation, oogenesis and reproduction. I. ricinus hosts a unique alphaproteobacterial symbiont, Candidatus Midichloria mitochondrii, which colonises the intermembrane space of host mitochondria, amplifies during blood-feeding and is vertically transmitted through oogenesis. Much about this symbiont remains unexplored, particularly how it invades mitochondria, whether it modulates mitochondrial cell-death pathways, and what benefits it confers to its tick host. Equally, the transcriptional landscape of I. ricinus ovary maturation and its intersections with symbiont dynamics during blood-feeding remains largely uncharacterised. Deciphering this molecular dialogue is essential for understanding how the symbiosis supports tick reproduction and pathogen transmission. This study combines deep transcriptomic profiling of I. ricinus ovaries at early, mid and late feeding stages with exome capture analysis of M. mitochondrii. Our results reveal extensive remodelling of host pathways governing cell-fate decisions, cytoskeletal organisation, extracellular matrix dynamics, immune defence, metabolism, reproduction and development to facilitate vitellogenesis. These changes are temporally coordinated with symbiont upregulation of flagellar and type IV secretion system genes during early feeding mechanisms likely underpinning mitochondrial invasion. These systems are absent in Midichloria strains unable to engage in intramitochondrial tropism, and their homologues are exploited by intracellular pathogens (e.g. Rickettsia) to breach host membranes, strongly indicating a similar invasion strategy. In addition, M. mitochondrii enhances transcription of ATP synthesising enzymes and vitamin/heme precursor pathways absent in ticks, while later activation of transposases suggests potential horizontal gene transfer. These findings illuminate a finely tuned, reciprocal transcriptional program underlies this pivotal symbiosis.

Keywords: Endosymbiosis, Mitochondria, Alphaproteobacteria, Exome-Capture

HFSP Reference Number: RGY0075/2017
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2017

MOUSE LEMUR FOR A NOVEL NEUROSCIENCE MODEL: A CELLULAR-RESOLUTION BRAIN ATLAS AND BEYOND

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The gray mouse lemur (Microcebus murinus), one of the smallest living primates, has emerged as a promising model organism for neuroscience research. Its genetic similarity to humans, evolutionary position between rodents and primates, and retention of many primate-specific features make it uniquely suited for studying the brain. At the same time, it retains rodent-like advantages, such as small size, short reproductive cycles, and experimental accessibility. While rodent models have been foundational in neuroscience, their evolutionary distance from primates limits their translational relevance to human applications. The mouse lemur presents a unique opportunity to bridge this gap, offering a powerful intermediary model. Notably, the gray mouse lemur is one of Madagascar's most abundant small native mammals, despite many lemur species facing critical conservation threats. Establishing the mouse lemur as a scalable neuroscience model requires comprehensive anatomical and functional mapping to enable meaningful cross-species comparisons of neural circuits.
To address this need, we developed eLemur, a digital framework consisting of a cellular-resolution 3D brain atlas that provides detailed anatomical, cytoarchitectural, and molecular insights into the mouse lemur brain. eLemur includes high-resolution brain-wide images immunostained with multiple cellular markers, allowing for precise delineation of cortical, subcortical, and other key brain regions. A comprehensive 3D cell atlas maps the spatial distribution and densities of neuronal and non-neuronal cell types, offering an essential resource for comparative neuroscience. The eLemur dataset is openly accessible via a web-based viewer (https://eeum-brain.com/#/lemurdatasets), facilitating data sharing and collaboration while integrating this primate model into broader neuroanatomical research. Building on this foundation, our research expands into the exploration of cell-type diversity, region-specific connectivity, and circuit-level organization, aiming to uncover how the mouse lemur’s brain architecture compares to that of rodents and larger primates. By integrating advanced high-resolution histology, molecular profiling, connectivity mapping, and fMRI, we aim to develop a multimodal framework for understanding primate cortical circuits. In collaboration with behavioral neuroscience researchers, we are correlating circuit organization with cognitive and sensorimotor behaviors to characterize how the mouse lemur processes information in comparison to well-established rodent and primate models. In conjunction with previous studies revealing hundreds of primate-specific gene expressions absent in mice, these anatomical findings highlight the mouse lemur’s unique position as an intermediary model between rodents and primates, with broader translational implications for understanding human brain function and associated disorders. With comprehensive anatomical mapping, functional circuit analysis, and behavioral collaborations, this research bridges the gap between traditional rodent models and primate and human neuroscience.

Keywords: Mouse Lemur, Brain, 3D Atlas, Cell type, web-based resource

HFSP Reference Number: RGP0024/2016
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2016

DEVELOPMENT AND VALIDATION OF INTERORGANELLE COMMUNICATION MATRIXES FOR STUDYING MITOCHONDRIAL NUCLEAR COMMUNICATION.

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In recent years, mitochondria became recognised as central regulators of cellular homeostasis functioning not only as energy producers but also as metabolic and signalling hubs. The ability of these organelles to respond to the needs of their host largely depends on their coordination with the nucleus. Indeed, the exchange of information between nucleus and mitochondria is fundamental for maintaining both cellular and mitochondrial integrity. This communication relies on signalling pathways defined by the direction of the information. Anterograde signals allow nuclear control over mitochondrial function and biogenesis, while retrograde signalling mitochondria conveys their functional status to the nucleus and triggers specific responses. Both anterograde and retrograde signalling become particularly relevant under conditions of stress and are crucial for the adaptive responses of cells to mitochondrial dysfunction and metabolic impairment. Despite significant progress in our understanding of the pathways underlying nuclear – mitochondrial communication, the molecular signals mediating this process remain poorly defined. Identifying the entity of the outgoing mitochondrial cues as well as the connection of each pathway to nuclear responses remains a major technical and conceptual challenge. An important obstacle in lies in the overlapping use of molecules in cellular signalling. Indeed, various cellular compartments constantly exchange information mainly using the same restricted number of signalling molecules. It is therefore extremely difficult to isolate a specific communication molecule and its specific effects from the ongoing signalling background. In the present work we exploit inorganic nature inspired matrixes to reconstitute in vitro a basic cellular unit composed by nucleus and mitochondria. We demonstrate that these nutritional matrixes can sustain the two organelles and allow their basic functions. Moreover, we develop specific protocols that allow single nucleus real-time measurements of second messengers and provide evidence of mitochondria-induced variations of nuclear Ca2+ signalling events. We conclude that inter-organelle matrixes could provide a means to disentangle the plethora of functions of specific signalling pathways, allowing the extrapolation of their roles in mitochondria nuclear communication.

Keywords: Nucleus, Mitochondria, Communication, Signalling, Matrixes

HFSP Reference Number: RG0024/2022
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

PROTEIN IMPORT INTO BACTERIA: HOW TELOMERE PHAGE TOXINS ENTER AND KILL KLEBSIELLA

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The cells of all eukaryotes including humans are characterized by subcellular organelles that import proteins encoded on nucleus-located genes, using elegant molecular machines in the organelle membranes to specifically and efficiently deliver cargo proteins. From the 1990s onwards it became clear how this protein import process worked for mitochondrial protein import. Evolution derived mitochondria from bacterial endosymbionts, making use of internal bacterial systems as a starting point for the innovations needed to manage protein assembly after they had been imported into mitochondria. The first step of this protein import system is a protein Translocase in the Outer membrane of Mitochondria, the TOM complex, and it has long been held that there was no ancestor to this complex because bacteria do not import proteins from their environment. My HFSP Long-Term Fellowship was devoted to discovery and characterization of the component parts of the TOM complex, and was recognized with a 10th Anniversary HFSP Award in 1999. My lab has since turned its attention to understanding how bacterial outer membranes are made and the nature of the topographical features that determine how bacteria compete in their environments. As part of those studies, we discovered a hugely prevalent group of bacteriophages (phages) in a genus of bacteria called Klebsiella. If a strain of Klebsiella carries one of these “telomere phages”, it will dominate the environmental niche over all other Klebsiella strains. The basis of this dominance is a new class of toxins called “telocins” encoded by the phage that are secreted by its host. The secreted telocin is lethal to Klebsiella that do not carry the phage. The mechanism of this killing depends on a new protein import system that all bacteria have, which is subverted by the toxin to gain entry into its prey.

REFERENCES

Hachiya N, Mihara K, Suda K, Horst M, Schatz G, Lithgow T. (1995) Reconstitution of the initial steps of mitochondrial protein import. Nature. 376:705-9. doi: 10.1038/376705a0

Shiota T, Imai K, Qiu J, Hewitt VL, Tan K, Shen HH, Sakiyama N, Fukasawa Y, Hayat S, Kamiya M, Elofsson A, Tomii K, Horton P, Wiedemann N, Pfanner N, Lithgow T, Endo T. (2015) Molecular architecture of the active mitochondrial protein gate. Science. 349:1544-8. doi: 10.1126/science.aac6428

Lithgow T, Stubenrauch CJ, Stumpf MPH. (2023) Surveying membrane landscapes: a new look at the bacterial cell surface. Nat Rev Microbiol. 21:502-518. doi: 10.1038/s41579-023-00862-w

Keywords: Mitochondria, Protein transport, Bacteria, Evolution

HFSP Reference Number: RG00203/1998-M
HFSP Award Category: Alumni Long-Term-Fellowship
HFSP Award Year: 1998

SHUFFLING THE GENOMIC DECK: ENGINEERING BACTERIAL GENOME DIVERSITY

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The ability to generate genomic diversity expands opportunities for understanding and engineering biology. We have engineered a molecular system that generates genomic diversity in bacteria, producing populations of cells with distinct genome architectures. This approach provides a framework for exploring both natural and synthetic genome design principles and for dissecting genome–phenotype relationships.

HFSP Special Invitee
Masterclass Australia 2025

UNLOCKING THE DIETS OF EARLY HOMININS: NEW METHODS AND INSIGHTS INTO THE ONSET OF MEAT CONSUMPTION AND BRAIN EXPANSION

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Dietary change, particularly the incorporation of animal resources, is considered a key driver of human evolution. Meat and other animal-based foods are nutrient-dense, energy-rich, and easily digestible, and are believed to have fueled brain expansion in our early ancestors. However, direct evidence of when and how regularly early hominins consumed animal resources remains limited. Although stone tools and cut-marked bones older than 3 million years provide some evidence, they offer little insight into the frequency or significance of meat consumption. Consequently, the timing and regularity of meat consumption by early hominins remain debated due to the lack of direct evidence.
Nitrogen isotope analysis in the collagen of bones and teeth provides valuable insights into diet by distinguishing between plant- and animal-based food sources. However, this method is only effective on relatively young fossils, as organic material in collagen degrades over time. Tooth enamel, by contrast, is highly mineralized and preserves mineral-bound organic material for millions of years, but its low nitrogen content has traditionally made isotope analysis challenging. To address this, we developed a biogeochemical method that enables nitrogen isotope analysis of mineral-bound nitrogen in enamel using only ca. 5 mg of material – over 100 times less than conventional methods require. We demonstrated that enamel nitrogen isotope values reflect diet and preserve a trophic signal, both in experimental and natural ecosystems. Applying this technique to Australopithecus teeth from the Sterkfontein Cave (between ca. 3.7 and 2.1 million years old), we found evidence of a variable but predominantly plant-based diet, with no isotopic indication of regular meat consumption in this small-brained, ape-like hominin.
In our ongoing HFSP project, we aim to advance isotopic studies of human paleodiet by measuring stable isotopic signals in intact amino acids preserved in tooth enamel. Using the Iso-Orbi technique – an innovative method combining electrospray-Orbitrap mass spectrometry with isotope analysis – we aim to investigate more specific dietary behaviors, such as distinguishing between herbivore and carnivore meat consumption, fish, fungi, and breastfeeding patterns. This approach may also allow us to explore whether early hominins hunted or scavenged, and whether cooking, which increases food energy density and may have supported brain expansion, was practiced. By integrating cutting-edge biochemical techniques with fossil evidence, this research has the potential to significantly enhance our understanding of how dietary changes influenced human evolution.

Keywords: Human Evolution, Diet, Amino Acids, Stable Isotope, Brain Development

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HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

SEEING IS BELIEVING: CORRELATED SINGLE-CELL IMAGING OF EXTRACELLULAR ELECTRON TRANSFER AND ASSOCIATED PROTEIN STRUCTURES REVEALS THAT DIVERSE ENVIRONMENTALLY IMPORTANT BACTERIA RESPIRE VIA CYTOCHROME NANOWIRES SECRETED BY HYBRID TYPE-4 PILUS/TYPE 2 SECRET

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Diverse species of anaerobic bacteria and archaea require long-range (>10 μm) extracellular electron transfer (EET) for respiration in the absence of soluble electron acceptors like oxygen. EET is involved in globally important environmental processes including biogeochemical cycling of carbon, nutrients and metals and bioremediation of toxic organic and metal contaminants in groundwater (1). Given the potential value of harnessing these processes for bioremediation, climate change, bioenergy, and bioelectronics, it is crucial to understand the mechanism of EET. Thousands of studies have claimed that diverse bacteria and archaea use e-pili, Type IV pili (T4P) made up solely of PilA-N, as nanowires for EET to (i) soil-abundant Fe(III) oxides, (ii) electrodes for high power generation and (iii) other species for consumption or production of methane (direct interspecies electron transfer, DIET) (1). However recent studies have challenged the role of e-pili in bacterial EET. Structural studies revealed that PilA-N assembles into a filament as a heterodimer with a second protein, PilA-C. The PilA-N-C heterodimer assembles into filaments that remain periplasmic during EET and form extracellular filaments only when artificially overexpressed. Furthermore, the extracellular filaments thought to conduct electrons have been identified not as PilA-N-based T4P but as cytochrome polymers. In Geobacter sulfurreducens (Gs) these nanowires are comprised of cytochromes OmcS, OmcZ or OmcE. However, until now, EET has not been directly demonstrated for either e-pili or cytochrome nanowires. To resolve this controversy, we correlated Geobacter filament heights measured using atomic force microscopy (AFM) with the atomic structures and biochemical analysis of the same filaments to establish filament composition unambiguously. Using a novel electron imaging method, we quantify single-cell EET to determine which of the numerous filaments function as nanowires. We find that cells produce and use OmcS and OmcZ nanowires, rather than pili or OmcE, for EET to Fe(III) oxides and electrodes. To characterize T4P and assess their role in EET we use complementary methods, including genetic engineering, biochemical analyses, bacterial two-hybrid assays, AFM and transmission electron microscopy imaging, and cryo-electron tomography to determine the structure of the pilus filament and its assembly machinery and identify the proteins involved in T4P biogenesis. We provide converging evidence that Gs T4P filaments do not serve as nanowires but rather combine components of T4P and type II secretion system (T2SS) to secrete cytochrome nanowires. Our study resolves a long-standing controversy regarding nanowire identity and function, establishing a new class of hybrid T4P-T2SS machinery that evolved for the secretion of cytochrome nanowires. These demonstrated design principles could enable control of microbial growth for bioremediation and methane reduction.

1. Yalcin, S. E., & Malvankar, N. (2020). The blind men and the filament: Understanding structures and functions of microbial nanowires Current Opinion in Chemical Biology, 59, 193-201. https://doi.org/10.1016/j.cbpa.2020.08.004

Keywords: Extracellular Electron Transfer, Cytochrome, Type 4 Pili, Type 2 Secretion Systems, Cryo-Electron Microscopy And Tomography

HFSP Reference Number: RGP017/2023
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

THE SWEET SOUND OF POLLINATION: IDENTIFYING PLANT RESPONSES TO VIBROACOUSTIC SIGNALS PRODUCED BY THEIR POLLINATORS

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Plants can perceive and respond to a wide range of environmental stimuli, from changes in light and temperature to more subtle cues such as volatiles and mechanical stimuli. However, their ability to detect and react to vibroacoustic signals has only recently gained attention. Previous studies have shown that plants exposed to vibrations generated by herbivores exhibit increased levels of defense-related compounds usually produced upon herbivory. Another study shows how the beach evening-primrose (Oenothera drummondii) rapidly increases brix degrees, an indirect measure for sugar concentration, of their nectar in response to pollinator flight sounds. To further investigate plant responses to acoustic stimuli, we have explored snapdragon (Antirrhinum litigiosum) natural hotspots in Valencia (Spain) and determined their most frequent pollinators throughout two seasons. Among these, we selected the solitary bee Rhodantidium sticticum and registered its acoustic signal and visitation pattern while hovering and entering A. litigiosum flowers. We subjected A. litigiosum plants grown under controlled phytotron and semi-field conditions to a three-hour playback of this primary pollinator's acoustic signal to mimic the visitation pattern, and compared it to pink noise, bumblebee playback, and silence treatments. Nectar was extracted for sugar concentration analysis, and flowers were collected for volatile profiling immediately after the first treatment and after five consecutive days of daily one-hour treatments. Results showed that plants exposed to pollinator sound exhibited significantly higher glucose, fructose, and sucrose amounts compared to those treated with pink noise, with stronger effects observed after five days. Total nectar volume was also affected. Additionally, floral transcriptomic analyses of the plants grown in phytotrons revealed differential gene expression in flowers and leaves, particularly in sugar transporter genes associated with nectar production. We are currently evaluating whether the treatments lead to differences in the volatilome composition of floral tissues, and we are also assessing the attractant properties of these emitted compounds. As side projects we are currently sequencing the genomes of A. litigiosum and R. sticticum to add resources for further studying transcriptomic responses in these two species. We are also developing deep learning methods to identify pollinator species based on their visitation acoustic patterns. Our findings add to the growing evidence that plants can perceive and respond to acoustic cues, suggesting a potential role for sound-mediated plant-pollinator interactions.

Keywords: Flower Pollination, Sound, Vibroacoustics, Buzzing, Pollinator

HFSP Reference Number: RGP0003/2022
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

ORIGINS OF CELL TYPES AND ALTERNATIVE NEURAL SYSTEMS

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The origin of animals and their cell types, including neurons, have been a central enigma since Darwin. Molecular reconstructions of ancestral toolkits are equally challenging due to poor representations of basal reference lineages. Nerveless Placozoa, with the simplest cellular organization without tissues, and elaborated ctenophores, with complex organs, two neural and several conductive systems, are the extreme examples of parallel evolution of the early-branching animals. Both phyla are critical for our understanding of the origin and evolution of metazoans. Here using single-cell multi-omics from different placozoan and ctenophore species with distinct ecology, we characterized distribution, functions, and phylogenomic relationships among basal cell types and transmitter systems identified in this study. First, our reconstruction predicts ancestral cell types in the Urmetazoan with subsequent parallel evolution and loss of some ancient cell lineages. We explore the scenario that neurons arose more than once from genetically different populations of secretory cells capable of volume transmission. Such primordial organization is currently found in Placozoa. We propose that injury-related signaling was the evolutionary predecessor for integrative functions of both ancestral secretory cell types and early transmitters such as glutamate, aspartate, glycine, NO, ATP, protons, and small peptides. These early signal molecules also acted as morphogenic factors with volume transmission that led to the formation of alternative integrative systems (in addition to a synaptic neural organization). Ancestral diversification of primordial secretory cells and signal molecules provided unique chemical micro-environments (chemo-connectomes) for behavior-driven innovations that pave the way to elementary cognition. Second, the earliest animal branch is ctenophores with complex organization, compared to bilaterians, but with independently evolved all representative metazoan cell types such as neurons, muscles, immune and digestive cells of through-gut. Most interesting, their simpler interconnecting neural and immune systems are chemically coupled, with reduced allorecognition, enabling designs and experimental bioengineering of hybrid nerve nets and even entire chimeric animals. The obtained neuro-bots and fused animals were able to be autonomous and survive, opening unprecedented opportunities for experimental 4D+ synthetic biology, from predictions and reconstruction of ancient neural circuits to making chimeric neural systems and chimeric animals.

Keywords: Animal Origin, Ctenophora, Placozoa, Nervous system evolution, Neurotransmitters

HFSP Reference Number: RGP0060/2017
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HFSP Award Year: 2017

DISCOVERY AND QUANTIFICATION OF AUTOCATALYTIC RNAS WITH GENERATIVE MODELS

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Estimating the plausibility of self-reproduction is central to origin-of-life scenarios. In particular, the RNA world hypothesis proposes that RNA was the molecular species that started evolution, acting both as a catalyst and an information carrier. Central to this scenario are self-reproducing RNAs. Self-reproduction may have started from autocatalytic RNAs that use oligomers as susbtrates, operating a rudimentary form of self-reproduction and evolution, which would have culminated in template-based replication of RNA catalyzed by RNA (replicase). Here, we address the initial stage of this process and more specifically the question of how common is autocatalysis among possible RNA sequences. Indeed, this property has been shown in only a handful of catalytic RNAs, which does not allow to assess the plausibility of starting life from random molecules. We combined machine learning and structure prediction algorithm, followed by high-throughput screening by sequencing. This allowed us to show the existence of a very large number of RNAs that assemble themselves from oligomers, covering a vast sequence space. We compared the generative power of models based on statistical covariation and secondary structure prediction. RNAs were found active as far as 65 mutations from the original Azoarcus sequence and up to 99 mutations away from each, far beyond the 10 mutations reachable by deep mutational scanning. Leveraging statistical physics methods, we computed the effective number of generated ribozymes to be ~1039 after correction by the experimental success rate. Sampled artificial ribozymes exhibited autocatalytic self-reproduction akin to the reference sequence. Using the fact that we have generated more sequences (>45.000) than have been detected in nature (~10.000), we also show how to further expand the diversity of molecules by relearning from positive and negative data, indeed allowing us to introduce >70 mutations in the original sequence, beyond the range explored by nature. Finally, we demonstrate the existence of scaling laws that control the probability of RNA structures and functions across varying lengths, which are empirically verified over known RNA families, including catalytic ones. Our findings show how the origin of life question can be addressed quantitatively and statistically rather than on the basis of singular instances. It provides quantitative laws to assess the plausibility of RNA functions. The redundancy of these functions seems typical, suggesting that abiogenesis could have followed a diversity of paths.

Calvanese, F., Lambert, C. N., Nghe, P., Zamponi, F., & Weigt, M. (2024). Towards parsimonious generative modeling of RNA families. Nucleic Acids Research, 52(10), 5465-5477. https://doi.org/10.1093/nar/gkae289

Lambert, C. N., Opuu, V., Calvanese, F., Zamponi, F., Hayden, E., Weigt, M., Smerlak, M. & Nghe, P. (2024). Expanding the space of self-reproducing ribozymes using probabilistic generative models. bioRxiv, 2024-07. https://doi.org/10.1101/2024.07.31.605758

Keywords: Origin Of Life; RNA; Generative Models

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HFSP Award Year: 2019

AVIAN SENSING OF AIRFLOW THROUGH FEATHER VIBRATION

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Many birds are extremely agile flyers and integrate multiple different sensory modalities to control their flight. Sensing the pattern of air over their wings is thought to be an important sensory input. Wing feathers vibrate in relation to the airflow over them and the wing has a rich array of mechanosensors within the wing close to the shafts of the feathers. However, very little is known about what information is transmitted by the feathers, encoded by the peripheral nervous system receptors and then processed in the brain. This multidisciplinary project looks at these three stages of information processing related to avian flight.
Using zebra finches (Taeniopygia guttata) as our study species we have characterised the relationship between airflow and feather vibration, for individual feathers and whole wings using acoustically driven vibration measurements and wind tunnel testing. We have found that feathers selectively transmit certain ranges of vibration frequencies and these filtering properties differ with wing extension or flexion.
In the peripheral nervous system, we have mapped the locations of the nerve endings thought to sense feather vibrations, the Herbst Corpuscles. We have built a three-dimensional reconstruction of the wing, identifying the distribution of the mechanoreceptor and their relationship to feather insertion sites. Finally, to identify areas of interest in the brain we first developed a quantitative description of flight behaviour and wing kinematics in bounding flights using high-speed videography and pose estimation in an arena with four different distances between perches. Flight trajectories become more stereotyped with experience for each distance, suggesting some form of learning over multiple flights. Comparison of observed flights with computational models reveals that, for these flights, birds do not appear to optimize a single parameter such as energy expenditure, speed, or bound duration, but rather select an intermediate trajectory, which may optimize a combination of flight parameters. Using a combination of electrophysiology and activity-dependent protein expression studies we have identified areas of interest for somatotopic mapping and, potentially, for learning. By combining approaches from aerospace engineering and neuroscience we are beginning to understand the flow of information from airflow through to the central nervous system and identifying the features of this information processing pathway used by the bird to select flight trajectories.

Keywords: Bird Flight, Feather Sensation

HFSP Reference Number: RGP0068
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

THE TRANSCRIPTIONAL PROGRAM OF GOLGI BIOGENESIS

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The transcriptional regulation of Golgi biogenesis has yet to be fully understood. Considering the fundamental importance of the Golgi complex for intracellular membrane trafficking, this dearth of knowledge represents a serious gap.
To solve this problem, we developed a reliable system to study de novo Golgi biogenesis. We engineered a cell line expressing HRP-tagged mannosidase II (ManII-HRP), which allowed us to destroy the preexisting Golgi with nano-surgical precision via HRP-mediated formation of insoluble DAB polymers in the Golgi lumen. Live cell imaging and electron microscopy demonstrated that such loss of the “old” Golgi induced the formation of the “new” fully functional Golgi complex in a substantial subpopulation of cells.
We combined this Golgi inactivation approach with single cell RNA-seq to analyze how the cell transcriptome changes during the process of de novo Golgi biogenesis. In particular, we addressed the following questions:
- Are genes encoding different components of the Golgi activated simultaneously or sequentially?
- How does the activation of certain groups of Golgi genes correlate with structural and functional changes in the rebuilding of the Golgi apparatus?
- Is there any order in the activation of genes encoding components of early (cis) or late (trans) Golgi sub-compartments, which differ from each other in structure, function, and composition?
We found that cells rebuilding a new Golgi apparatus transactivate more than 100 Golgi genes encoding various classes of Golgi components, including structural proteins, glycosylation enzymes, and membrane tethering/trafficking complexes. The upregulation of these genes occurred in a coordinated manner and coincided temporally with the reappearance of Golgi units exhibiting the typical stacked architecture, as well as with the reactivation of transport through the newly forming Golgi organelle. Notably, the activation of Golgi genes was not compartment-specific, as the induction of cis-, medial-, and trans-Golgi genes occurred simultaneously. We next investigated which transcription factor(s) (TFs) drive this massive transactivation of Golgi genes to support Golgi biogenesis. By combining TRANSFAC bioinformatics analysis with subsequent genetic screening, we identified three TFs, CREB3L1, SP1, and MITF, as key regulators of this process. Silencing of these TFs significantly impaired both the induction of Golgi genes and the biogenesis of the new Golgi apparatus. Furthermore, we found that this transcriptional mechanism operates in a physiological context. It was activated during plasma cell differentiation, a process in which the Golgi apparatus expands sevenfold relative to parental B cells to accommodate increased IgG glycosylation and secretion. Together, our findings suggest that the coordinated transactivation of a broad array of Golgi genes drives de novo Golgi biogenesis by providing the structural and functional components required for the formation and activity of the growing Golgi organelle.

Keywords: Golgi Complex, Organelle Biogenesis, Transcriptional Regulation, Nano-Surgery, Scrna-Seq

HFSP Reference Number: RGP0046/2021
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2021

UNRAVELING THE MECHANISMS OF MOLECULAR RESILIENCE OF NEURONS TO CYCLIC MECHANICAL STIMULATION

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Neurons are constantly exposed to mechanical forces throughout development, daily activities, and pathological conditions. While high-intensity mechanical stress can be detrimental, low-intensity stimuli may contribute to neuronal resilience and adaptation. A key player in this process is the microtubule (MT) cytoskeleton, which responds dynamically to mechanical cues. However, the effects of repetitive mechanical compression over a lifetime remain poorly understood.
In this study, we investigated the impact of repetitive mechanical strain on dorsal root ganglion (DRG) neurons by applying cyclic compression at varying intensities (2.5%, 5%, and 10%). Our findings reveal a strain-dependent neuronal response. At 10% strain, neurons undergo severe cytoskeletal damage, leading to loss of organelles, axonal degeneration, and ultimately, cell death. In contrast, low strain (2.5%) does not impair cell viability but induces axon retraction, accompanied by an increase in MT stability—suggesting a protective response promoting axonal repair. Interestingly, an intermediate strain (5%) initially disrupts MT architecture, leading to axon retraction and cytoskeletal disorganization. However, neurons activate a recovery mechanism, restoring MT stability and axon length within 24 hours. Transcriptomic analysis reveals that the RAS signaling pathway plays a central role in this recovery, suggesting a molecular response to mechanical stress that enables neurons to reorganize and maintain their structural integrity.
These findings indicate that neurons exhibit a threshold-dependent response to mechanical strain. While high strain leads to irreversible damage, low and intermediate strain levels activate adaptive mechanisms that support MT stabilization and axonal regrowth. This study advances our understanding of neuronal mechanotransduction and highlights the intrinsic resilience of neurons to mechanical challenges. Understanding these adaptive responses could provide new insights into neurodegenerative conditions and potential therapeutic strategies for enhancing neuronal repair.

Keywords : Microtubule, Cyclic Compression, Neuron, Resilience

HFSP Reference Number: RGP0026/2021
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2021

GLI1-EXPRESSING STROMAL CELLS ARE HIGHLY REPARATIVE PRECURSORS OF LONG-LIVED CHONDROPROGENITORS IN THE FETAL MURINE LIMB

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How is the number of progenitor cells in a growing organ controlled to sustain a species-specific tissue architecture and growth rate? While dozens of genes/proteins that control cell size and number are known, how they coordinate to achieve a robust outcome, even in the face of developmental perturbations, is still a mystery. The growth-plate cartilage of the developing long bones is a well-known system of spatially segregated stem/progenitor, transient amplifying and terminally differentiated cells. However, the regulation of the number and activity of long-lived cartilage progenitors (LLCPs) is poorly understood, despite its relevance for understanding human-height variation, the evolution of limb size and proportions and the aetiology of skeletal growth disorders. Moreover, whether their behaviour can adapt to developmental perturbations, generating robustness, has not been explored. Here, we show that Gli1+ cells are the fetal precursors of postnatal LLCPs, and that Gli1+ LLCP precursors remain mostly dormant until postnatal stages. However, in response to cell-cycle arrest targeted to the fetal-cartilage, they expand in the cartilage, enabling normal growth. We further show that reparative Gli1+ cells originate from Pdgfra+ cells outside the cartilage, revealing the surrounding tissues as an unexpected CP source. Elucidating how stromal cells become Gli1+ LLCPs could shed light on developmental robustness and lead to growth-boosting therapies

Keywords: Limb Development, Endochondral Ossification, Catch-Up Growth, Mouse Genetics, Developmental Robustness

HFSP Reference Number: CDA00021/2019-C
HFSP Award Category: Alumni Career Development Award
HFSP Award Year: 2019

INTEGRATED APPROACHES FOR THE RECOGNITION OF SMALL MOLECULE INHIBITORS FOR TOLL-LIKE RECEPTOR 4

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Toll-like receptors (TLRs) are pattern recognition receptors present on the surface of cells playing a crucial role in innate immunity. One of the TLRs, TLR4, recognizes LPS (Lipopolysaccharide) as its ligand leading to the release of anti-inflammatory mediators as well as pro-inflammatory cytokines through signal transduction and domain recruitment. TLR4 homodimerizes at its intracellular TIR (Toll/interleukin-1 receptor) domain that helps in the recruitment of the TRAM/TICAM2 (TIR domain-containing adaptor molecule 2) molecule. TRAM also contains TIR domain which in turn, dimerizes and functions as an adapter protein to further recruit TRIF/TICAM1 (TIR domain-containing adaptor molecule 1) protein for mediating downstream signaling. Apart from LPS, TLR4 also recognizes endogenous ligands like fibrinogen, HMGB1, and hyaluronan in autoimmune conditions and sepsis. We employed computational approaches to target TRAM and recognize small molecule inhibitors from small molecules of natural origin, as contained in the Super Natural II database. Finally, cell reporter assays and NMR studies enabled the identification of promising lead compounds. Hence, this study aims to attenuate the signaling of the TLR4-TRAM-TRIF cascade in these auto-inflammatory conditions.
Paper corresponding to this work can be obtained from https://doi.org/10.1016/j.csbj.2023.07.026.

Keywords: Virtual Screening, Drug Design, Immunity, Sepsis, Protein-Protein Interactions

HFSP Reference Number: RGP0054/2009-C
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2009

APHROGUT: DISSECTING THE PROCESS LEADING TO YEAST MATING IN SOCIAL WASP INTESTINES

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A strong natural association between Saccharomyces cerevisiae and social wasps supports the yeasts' persistence, spread, and evolution through sexual reproduction. Mating between different S. cerevisiae strains, an event never observed in natural settings, occurs in the social wasp intestine, which might provide the ideal conditions for this process. However, the limited understanding of social wasp physiology and yeast mating in natural environments hinders a full appreciation of the implications and mechanisms of this close relationship. Thanks to a multi-disciplinary team, including chemists, systems biologists, physicists, entomologists, and microbiologists, we could properly address this multifaceted system in all its key aspects. The inter-strain mating process involves several stages: natural yeast strains, mostly diploid, undergo meiosis (sporulation) under stressful environmental conditions, forming four sexual haploid spores within a robust wall (ascus). Inter-specific strain mating can only occur if the sexual spores are released from the ascus through enzymatic or physical stress and, after germinating in suitable conditions, encounter spores of other strains and the opposite mating type. A critical starting point was defining the chemical and physical characteristics of the social wasp intestine, which have been poorly described so far but are fundamental for understanding the features promoting inter-strain S. cerevisiae mating. To achieve this, customized chemical probes were designed, synthesized, and tested to quantify the chemical properties of the two main compartments: the social crop and the gut. These compartments differ in pH, viscosity, and nutritional availability, thus exposing yeast cells to a range of environments that, as confirmed by in vitro analyses, can promote sporulation and spore germination. Furthermore, although in vitro evaluation of shear stress revealed that wasp intestinal peristalsis is not sufficient to break the yeast ascii and release the spores, a combination of genetic analyses, host transcriptional profiling, protein structure prediction, and in vitro analyses highlighted the expression of host enzymes capable of promoting ascus lysis. To assess the likelihood of spores germination (and hence mating), a pool of yeast strains deleted in essential and non-essential genes was exposed to the wasp intestine environment, allowing for the identification of genes necessary for yeast survival in this context. Additionally, transcriptional analysis of spores ingested by wasps provided molecular evidence of germination. Understanding the factors that shape yeast ecology requires extensive multidisciplinary collaboration. Exploring yeast-insect associations continues to yield valuable insights in supporting this complex endeavor.

Keywords: Yeast, Social Wasps, Chemical Probes, Microrheology, Model Of Molecular Pathways

HFSP Reference Number: RGP0060/2021
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2021

HOW ANIMALS PRODUCE VIVID COLOURS: NEW INSIGHTS FROM REPTILES

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Colours in nature are produced by exploiting universal optical principles, but there is an extraordinary diversity of variations on fundamental themes, which provide rich grounds for new discoveries. In vertebrates with coloured skin, such as reptiles, amphibians and fish, colour is produced by a system of layered pigment cells which contain absorbing pigments or reflective crystals. These cells have been studied for many decades; but there are significant gaps in our understanding of how absorbing and reflective components interact to produce vivid colours, and about their molecular development and control. Here we share some new insights about how colour is produced in the skin of reptiles. We show that 'colourless' pteridine pigments dominate in reptile skin and correlate strongly with colour saturation. The contribution of these colourless pteridines to observed skin colour is an enduring puzzle and we discuss insights into their role from our own and previous work. We present evidence that rapid, reversible colour change from red to yellow is enabled by motility of vesicles containing red pteridine pigment in xanthophore cells, a phenomenon previously unknown in reptiles. Our analyses also highlight the central and underappreciated role of riboflavin (vitamin B2) as a yellow colorant in the skin of reptiles, particularly snakes. Together, these insights contribute to our overarching goal of developing a comprehensive and generalisable model of colour production in the skin of reptiles.

Keywords : Pigment Cell, Xanthophore, Ultrastructure, Pteridines

HFSP Reference Number: RGP0037/2022
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

T CELL MICROVILLUS AS A SIGNALING ORGANELLE

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The surfaces of many immune cells, including T cells, are densely covered with submicron-sized membrane protrusions known as microvilli. Traditionally, microvilli have been associated with lymphocyte migration and adhesion. However, recent studies suggest that they also play a critical role in the enrichment and organization of key T cell signaling molecules, although the mechanisms and functional implications of this enrichment remain inadequately understood.
In this study, we explored the geometric regulation of signaling molecule organization within microvilli using a multidisciplinary approach. Our approach combined live-cell super-resolution microscopy (STED), nanofabrication, and in vitro reconstitution of microvilli-like compartments. Upon antigen engagement, we observed a rapid clustering of specific receptors at microvilli-target cell contact sites, accompanied by the exclusion of the phosphatase CD45. This was followed by the recruitment of the kinase ZAP70 and the condensation of LAT, a key adaptor protein critical for effector assembly, highlighting a dynamic signaling cascade localized within these structures.
To dissect the mechanism underlying the enrichment of LAT condensates in microvilli, we engineered nanostructures with precisely defined curvatures. Remarkably, LAT condensates preferentially accumulated in concave (negative) curvatures with radii approximately 400 nm, while avoiding convex (positive) curvatures. These findings reveal a novel role for protein condensates in sensing mesoscale membrane geometry, expanding the classical paradigm where curvature sensing was believed to be mediated solely by single structured domains such as BAR.
Furthermore, our results identified microvilli as hotspots for calcium signaling, consistent with their role in amplifying early activation signals. Collectively, our work provides a high-resolution map of protein dynamics within microvilli, demonstrating how their unique geometry facilitates signal transduction through the spatial regulation of molecular condensation. These findings establish microvilli as specialized signaling organelles that play a critical role in mediating T cell activation.

Keywords : T Cell, Microvilli, Condensation, Super Resolution, Nanostructure

HFSP Reference Number: RGY0088/2021
HFSP Award Category: Alumni Research Grant Early Career
HFSP Award Year: 2021

UNRAVELING THE TRANSCRIPTOMIC LANDSCAPE OF REPTILIAN CHROMATOPHORES: FROM DEVELOPMENT TO COLOR-PRODUCING CELLS

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Reptiles exhibit an extraordinary diversity of skin colors and patterns, far surpassing that of mammals. This diversity results from the spatial arrangement of pigmentary and structural color cells, known as chromatophores, within their scales. Melanophores and xanthophores contain black and yellow/red pigments, respectively, whereas arrays of guanine nanocrystals within iridophores produce a wide range of colours through light interference. Although the pigment content, distribution and transcriptomic profile of post-embryonic reptilian chromatophores have already been discussed, little is known on the development of these cells.
To address this gap, we undertook a large-scale single-cell transcriptomic study to elucidate the dynamic gene expression profile of these cells as they differentiate from neural-crest cells and migrate within the embryonic skin to establish the spectacular color patterns seen in hatchlings. We generated the molecular profile of each chromatophore lineage in two Squamate model species: the corn snake and the bearded dragon lizard. We sampled the embryonic skin at eight and seven developmental stages, respectively, and we sequenced the transcriptomes of disassociated cells.
For each species, we identified distinct chromatophore cell clusters and gene markers that characterise them by their specificity. Furthermore, we traced the developmental trajectories of each cell type to maturity. This allowed us to pinpoint the commonalities in Squamate chromatophore differentiation and uncover marked differences between the two species. In addition, we performed whole mount in situ hybridisations with species- and cell type-specific probes to shed light on the patterning dynamics during development, at stages when the pigment production has not yet begun.
We also compared the gene expression profile of reptilian chromatophores to those of zebrafish. Reptiles have a direct development, whereas the zebrafish goes through metamorphosis during which melanophores and iridophores de novo differentiate from progenitor cells, while xanthosomes become cryptic and newly acquire their pigmentation at the end of the process. We investigated whether the corn snake chromatophores are more similar to the pre- or post-metamorphotic zebrafish chromatophores. Additionally, we also compared them to chromatophores from Xenopus frog. These comparisons provide a transcriptomic framework to understand the molecular mechanisms that govern coloration in ectothermic vertebrates.
Overall, these findings not only advance our understanding of chromatophore development in reptiles but also offer valuable insights into the evolutionary and molecular mechanisms that shape the vibrant coloration seen in ectothermic vertebrates.

Keywords: Skin Coloration, Chromatophores, Reptilian Development, Single-Cell Transcriptomics, Squamates

HFSP Reference Number: RGP0037/2022
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

REGULATION OF ADIPOCYTE HYPERTROPHY AND ITS IMPACT ON SYSTEMIC METABOLISM

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At times of energy surplus, most excessive energy is stored in white adipocytes in form of triglycerides. This makes white adipose tissue the primary site of energy storage. Increased lipid storage can be either facilitated by hyperplasia, the storage of fat in de novo differentiated adipocytes, or hypertrophy, the storage of fat in already existing adipocytes. Hypertrophic adipose expansion can exceed the lipid storage capacity of adipocytes, resulting in adipocyte death, inflammation and spillover of lipids to other organs such as the liver and skeletal muscle. Local and systemic inflammation in combination with ectopic lipid accumulation can then initiate the development of insulin resistance and the metabolic syndrome. Thus, understanding the mechanisms of adipocyte lipid accumulation and hypertrophy is important to develop novel therapeutic approaches dissociating obesity from the metabolic syndrome. We show that increased basal insulin secretion from pancreatic beta cells, not reaching the threshold to induce tissue glucose uptake, suppresses adipocyte lipolysis resulting in adipocyte hypertrophy in chow diet fed animals. These lean chow diet fed mice showed improved glucose tolerance compared to control animals. However, feeding a hypercaloric high fat diet, to these mice, with already enlarged adipocytes, accelerated the development of insulin resistance and glucose intolerance. Thus, natural variations in basal insulin secretion could have profound effect on the predisposition for the development of metabolic disease in context of a hypercaloric diet. Moreover, we show that the small cell surface protein Nrac is a regulator of CD36 mediated fatty acid uptake. Loss of Nrac accelerates adipocyte fatty acid uptake leading to adipose hypertrophy. Importantly, loss of Nrac at the cell surface occurs naturally in response to increased extracellular fatty acid concentrations. Thus, we identified a novel regulatory mechanism limiting adipocyte fatty acid uptake at times of low circulating fatty acid concentrations. This allows utilization of fatty acids in organs such as the heart, requiring fatty acids for energy production. Conversely, to prevent lipotoxicity, when circulating fatty acid levels are high, loss of cell surface localization of Nrac results in elevated fatty acid influx of adipocytes for storge. Overall, we reveal important mechanism of both lipid uptake and lipolysis in the regulation of adipocyte hypertrophy.

Keywords: Obesity, Insulin resistance, Adipocyte, Metabolism, Physiology

HFSP Reference Number: 2009-2012
HFSP Award Category: Alumni Long Term Fellowship
HFSP Award Year: 2012

HAEMOGENIC AND INTERMEDIATE GASTRULOIDS: NOVEL 3D STEM CELL-BASED EMBRYO MODELS TO STUDY BLOOD DEVELOPMENT IN VITRO

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During embryonic development of mammalian organisms, blood progenitors are formed in three sequential waves. The first two waves happen in extra-embryonic tissues, such as the yolk sac. They produce embryonic blood, which for the most part is transient and does not persist postnatally. The third wave of embryonic blood development occurs in the large intra-embryonic arterial blood vessels. Alongside transient progenitors, the third wave uniquely generates haematopoietic (i.e., blood) stem cells (HSCs), which persist lifelong. HSCs are formed in clusters that bud off from the inner wall of the dorsal aorta, specifically in the part of the aorta that runs through the Aorta-Gonad-Mesonephros (AGM). The AGM is an embryonic structure that also forms the reproductive organs ('gonads') and embryonic kidneys ('mesonephros').
The formation of embryonic blood, and of HSCs, is regulated by signals produced by their embryonic niches. However, due to the relative inaccessibility of the mammalian embryo, which develops inside of the uterus, and the limited cell numbers that can be obtained from blood-forming niches, our knowledge of embryonic blood development, and particularly the role of the niche, remains incomplete.
Here, we sought to address niche contributions to blood formation by developing mouse embryonic stem cell (ESC) -based 3D embryo gastruloid models, which reconstruct extra- and intra-embryonic blood-forming niches in vitro with spatial and temporal accuracy. Our new gastruloid models were generated by treating aggregates of mouse ESCs with defined levels of Wnt and Nodal/TGF-beta signals, which induces a robust symmetry-breaking and self-organization process that transforms the aggregates into spatially organized embryo-like structures. Through in situ hybridization, immunofluorescence, flow cytometry, single-cell and bulk transcriptomics, and live-imaging, we show that our new gastruloid embryo models (1) generate putative yolk sac-like and AGM-like structures, and (2) capture time-dependent specification of embryonic blood progenitors, which recapitulate migration patterns observed in vivo. Significant to blood formation, we observed that the balance between Wnt and Nodal/TGF-beta signals alters tissue specification within the mesoderm, the embryonic tissue layer from which the blood and blood-forming niches originate, and that modifying the relative concentrations of these signals fine-tunes the cellular composition of the blood niches in our gastruloids. Altogether, our new gastruloid models can be used to study embryonic blood formation in vitro and to specifically address the role of signals from the 3D niche in blood formation. Generation of the AGM, which is the niche for adult HSCs, provides an essential cornerstone for future in vitro generation of clinically relevant HSCs, which could be used for bone marrow transplantations to treat a wide variety of blood-related diseases.

Keywords: Blood Development, Embryology, 3D Stem Cell-Based Embryo Models (Gastruloids), Haematopoietic Stem Cells, Embryonic Organoids

HFSP Reference Number: LT0047/2022-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2022

SINGLE MOLECULE LOCALIZATION IMAGING OF ENV CLUSTERING IN NATIVE HIV-1 VIRUSES

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Viral envelope proteins are essential for mediating virus entry into host cells. Typically existing as glycosylated trimers, these proteins undergo significant conformational changes during receptor binding and membrane fusion. In HIV-1, the envelope glycoprotein (Env) trimers are critical for infection of CD4+ T cells and macrophages. Super-resolution fluorescence microscopy, such as STED, has revealed that Env is not randomly distributed but instead forms distinct clusters on the surface of mature HIV-1 virions—structures thought to be key for viral entry and immune targeting. Here, I will present high-resolution visualizations and quantitative analyses of HIV-1 Env clusters using DNA-PAINT, a single-molecule super-resolution fluorescence microscopy technique capable of achieving 10 nm resolution. To preserve Env's structural and functional integrity, we used AlphaFold2 to guide the insertion of a synthetic epitope at an optimal site, allowing precise labeling with high-affinity single-domain antibodies (sdAbs) without disrupting native conformation. Our findings provide novel insights into the spatial organization of Env on both mature and immature HIV-1 virions. Notably, DNA-PAINT imaging enables the visualization of Env trimers in both open and closed conformations—key states for viral entry. These results enhance our understanding of Env microclusters and their role in HIV-1 biology, highlighting their importance as potential targets for immune responses.

Keywords: HIV-1, Single-molecule localization microscopy, DNA-PAINT

HFSP Reference Number: LT0025/2023-C
HFSP Award Category: Awardee Cross Disciplinary Fellowship
HFSP Award Year: 2023

THE CELL BIOLOGY OF PLURIPOTENCY

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The transformation of a mammalian embryo from a tiny soccer ball-like structure into a newborn with four limbs, a beating heart and big bright eyes is one of the most remarkable and fundamental processes of life. Inside the soccer ball-like embryo resides a handful of “all-rounder” cells, known as pluripotent cells, which can give rise to any type of cell in the adult body. Enigmatically, the capacity for pluripotency is lost in the embryo less than one week after conception. But why? Knowing precisely how pluripotency is controlled can transform the way we think about how cells behave during development and regeneration.
Until recently the inimitable potency of pluripotent cells has been known to be regulated by a combination of genetics, epigenetics and external factors. We were the first to discover that pluripotent cells develop and mature into distinct cell types also based on the functions of their inner scaffolding, known as the microtubule cytoskeleton, which was until then widely regarded as disorganised and its contribution to cell fate specification was largely ignored. These seminal discoveries led to the establishment of a novel sub-field in developmental and stem cell research, focussing on the “cell biology of pluripotency”.
Inside a cell, organelles and proteins are usually not randomly distributed but are assigned to regions where they are needed. The cell utilises the microtubule cytoskeleton as the road map to localise organelles and to trigger the relay of signals intra- and intercellularly. By performing innovative live imaging of preimplantation mouse embryos and human induced pluripotent stem cells, we discovered an unprecedented form of non-centrosomal microtubule organisation required for the formation and maintenance of pluripotency. Dependent on the microtubule anchor and nucleator Calmodulin-Spectrin associated protein 3 (CAMSAP3), this form of non-centrosomal microtubule organisation orchestrates the asymmetric distribution of cell adhesion proteins, RNAs and organelles inside pluripotent cells which results in an unequal inheritance of information, and differential cell fate decisions of daughter cells. By applying innovative light-switchable microtubule destabilisers, we demonstrated the inability of pluripotent cells to specify their fates due to failing in establishing intracellular asymmetries.
Our discoveries comprehensively address the cell biological fingerprint of pluripotency during mammalian embryogenesis and challenge the pluripotency dogma that dynamic regulation of the intracellular organisation, orchestrated by the microtubule cytoskeleton, is critical for this feature. By addressing the knowledge gap in the cell biological properties of pluripotent cells, we can harness this knowledge to develop novel therapeutics for regenerative medicine and fertility.

Keywords: Pluripotency, Embryogenesis, Cytoskeleton, Microscopy, Regenerative and Reproductive Medicine
HFSP Reference Number: LT000164/2015
HFSP Award Category: Alumni Long-Term-Fellowship
HFSP Award Year: 2015

POSTER TEASER TALKS

A FUNCTIONAL GENOMICS APPROACH TO STUDY HUMAN-SPECIFIC NEURAL PROGENITOR CELL DEVELOPMENT

Mai Ahmed[1]; Ahmed Moselhi[2]; Aditi Aggarwal[2]; Wendy Choi[2]; Graham Macleod[3]; Xiaoxue Dong[1]; Kevin Brown[1]; Patricia Mero[1]; Jason Moffat[1]; Stephane Angers[3]; Julien Muffat[1]; Yun Li[1]
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Outer radial glia (oRG) is a recently identified population of neural progenitors that is highly enriched in the brains of humans and higher primates. oRG generate the majority of human cortical neurons and play key roles in cortical expansion and folding. Recent studies have shown that defects in oRG development are implicated in the pathology of several neurodevelopmental disorders. However, studying oRG development has been challenging because of the inaccessible nature of the human fetal brain. In this study, we utilized human pluripotent stem cells to model oRG in vitro. To determine the mechanisms that regulate oRG expansion during development, we performed a genome-wide CRISPR knockout screen in oRG-like cells. Our screen identified hundreds of genes that perturb oRG behaviour, many of which are linked to neurodevelopmental disorders, such as autism. Our findings shed valuable insights into the genetic factors that govern oRG development and survival, offering a deeper understanding of the mechanisms underlying the pathology of various neurodevelopmental disorders.

Keywords: CRISPR Gene Editing, Neural Progenitor Cells, Neurodevelopmental Disorders

HFSP Reference Number: LT0042/2023
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

USING OPTOGENETICS TO INVESTIGATE EXTRACELLULAR MATRIX-ENCODED SIGNALS THAT ORCHESTRATE FIBROBLAST HETEROGENEITY

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Fibroblasts are one of the key cell types that deposit and remodel extracellular matrix (ECM), conferring structure to tissues and organs. In pathologies like cancer and fibrosis, quiescent fibroblasts get activated and give rise to heterogenous cell states, including myofibroblasts (myFibs) and immune-modulatory fibroblasts (iFibs). Several biochemical cues that modulate fibroblast activation have been identified, including TGFß, FGF, and NFκB signalling pathways. However, the cell-matrix mechano-chemical feedback interactions that lead to the emergence of these fibroblast states, as well as how these activation signals propagate in space and time is unclear. In this study, we use spatio-temporal optogenetic manipulation of fibroblast states to address these questions. To this end, we have established normal skin- and lung-derived fibroblast cell lines with alpha Smooth Muscle Actin (αSMA:DsRed) and Interleukin-6 (Il6:iRFP682) fluorescent reporters to read out myoFib and iFib states, respectively. To manipulate fibroblast activation by light, we introduced the previously published OptoTGFBRs (Li et al., ACS Synth. Biol. 2018) or OptoFGFR (Grusch et al., EMBO J. 2014) or OptoTraf6 (DeFelice et al., Sci. Signal 2019). First, we validated that blue light exposure triggered an immediate activation of their respective pathway-specific reporters. OptoTGFBR activation led to the nuclear translocation of Smad2-iRFP682 reporter, while OptoFGFR activation led to cytoplasmic translocation of the ERK-KTR-iRFP713 reporter, and finally OptoTraf6 activation led to the nuclear translocation of P65-iRFP682 reporter. Next, upon long-term light stimulation, OptoTGFBR cells activated myoFib fate and downregulated iFib fate, as assessed by the αSMA and Il6 reporters. On the other hand, both OptoFGFR and OptoTraf6 lines downregulated myoFib fate and upregulated iFib fate. In addition, fibroblast-derived matrix (FDM) assays showed that all three Opto systems secrete and remodel diverse kinds of ECM. Strikingly, quiescent fibroblasts freshly plated over these Opto FDMs activated their respective ancestral Opto cell fates. Specifically, quiescent fibroblasts plated over OptoTGFBR FDM activated αSMA reporter, while the ones plated over OptoFGFR FDM downregulated the same. These data show that ECM-encoded signals alone can contain and transmit cell state information. Currently, to identify the cell-matrix feedback loops involved in this phenomenon, we are characterising the composition, spatial organization, and mechanical properties of these matrices. Altogether, this work defines the heterogeneity in fibroblast activation states, and how the activation signals can be encoded in the ECM they secrete and remodel.

Keywords: Fibroblasts, Optogenetics, Heterogeneity, Extracellular matrix, Feedback loops, Cell states

HFSP Reference Number: LT0010/2023-L1
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HFSP Award Year: 2023

BUILDING A SINGLE CELL EPIGENETIC OSCILLATOR

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DNA packaging proteins, histones undergo covalent modifications to regulate gene expression. These modifications can facilitate the activation or repression of a gene. In-turn the dynamics of the modifications are regulated by enzymes that 'write', 'read' and 'erase' these modifications. Here, we theoretically describe an epigenetic oscillator, and show our preliminary experimental results. Our design has the enzyme that writes repressive modifications under its own control effectively a ‘self-repressor’, providing strong negative feedback essential for oscillatory systems. There is inherent delay in the circuit which requires the signal to be transmitted from the DNA sequence to functional proteins, and back writing/spreading the epigenomic modifications to establish an OFF state. Once repressed, DNA replication and enzymatic erasure of these modifications contribute to driving the cells to ON state. Building a clock at the single cell level facilitates systematic investigation of the components of feedback and sources of delay providing fundamental insights into epigenetic mechanisms. For instance, varying the genomic position of the enzyme that writes repressive modification relative to its nucleation site, we can quantitatively study the spatial dynamics of epigenomic mark propagation contributes to the delay thus affecting the oscillator behavior.

Keywords: Histone modifications, Negative feedback

HFSP Reference Number: LT0020/2024
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2024

IDENTIFYING MOLECULAR DETERMINANTS OF GLOBAL EPISTASIS AND PROTEIN SUPERBINDERS BY ACCURATELY SCREENING AND QUANTIFYING COMBINATORIAL DEEP MUTATIONAL SCANNING LIBRARIES.

Pau Creixell

Decreasing costs in DNA synthesis and sequencing and computational resources enabling the desiing, screening and analysis of combinatorial deep mutational scanning libraries (CDMS), where all mutagenized sites in a protein are tested in all possible combinations with each other. Beyond traditional deep mutational scanning (DMS), CDMS allows us to study non-linear context dependencies, also known as epistasis, and the screening of an exhaustive protein space to identify proteins with new improved functions without the mutational biases of functional intermediates' requirements of directed evolution. However, fulfilling these benefits requires a new analytical framework capable of accurately quantifying global epistatic terms. Here we develop and test such global wild-type free approach by screening WW domains using a phosphoserine-encoding (21-amber) oligo library, displayed on bacteria, and demonstrate 45-80% improved predictive performance and identify molecular determinants of global epistasis and protein superbinders that we structurally resolve. Altogether, we establish that natural proteins recognize binders non-linearly, that mutating determinants of epistasis suppresses this function, and that global approaches enable better design and understanding of protein bingers for future biochemical, synthetic biology and drug discovery applications.

Keywords: Protein Biochemistry, Epistasis, Combinatorial DMS Libraries

HFSP Reference Number: RGEC27/2024
HFSP Award Category: Awardee Research Grant Early Career
HFSP Award Year: 2024

mRNA DISPLAY PIPELINE FOR PROTEIN BIOSENSOR CONSTRUCTION

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Protein biosensors hold great promise for diagnostics and research, but their development remains largely empirical. To streamline this process, we established a modular biosensor architecture, and a rapid binder selection pipeline based on mRNA display. Our workflow enables the generation of hyper-stable monobody binders (FN3con) that can be readily integrated into a two-component allosteric biosensor, utilizing a calmodulin-reporter chimera.cWe validated this system by developing biosensors against clinically relevant targets including cytosolic aspartate aminotransferase (a liver toxicity marker), and heart failure biomarkers such as S100A7 and Galectin-3. Our pipeline consistently yielded over 1,000 unique binders per target within one week. Interestingly, we found that binder affinity alone did not predict biosensor performance. Interactions between the binding domain and reporter module significantly influenced both activity and dynamic range. These findings underscore the importance of multiplexed biosensor prototyping following binder selection to identify optimal constructs.

Keywords: Biosensor Development, Mrna Display, Monobody, Biomarkers

HFSP Reference Number: RGP0002/2018
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2018

IS QUANTUM COHERENCE IMPORTANT IN COUPLING THE ANTENNA SYSTEM TO THE PHOTOSYSTEM IN CRYPTOPHYTES?

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Cryptophytes, unicellular eukaryotic algae found in marine and freshwater environments, are characterized by their distinct red or blue-green coloration, owing to unique pigments such as chlorophylls, as well as phycobiliproteins (PBPs). Thriving in environments with limited photon availability, cryptophytes have evolved a remarkably efficient light-harvesting process. Their light-harvesting system features an antenna structure inherited from cyanobacteria, composed of PBPs. This antenna expands the absorption area and spectral range for incoming photons. Upon photon absorption, an exciton—an electron-hole pair—is generated. The efficient transfer of this exciton from the antenna to the photosystem is essential for photosynthesis. Spectroscopic evidence reveals the involvement of quantum coherence in this transfer process, as indicated by quantum beats. However, the challenge lies in discerning the true source of this quantum coherence among multiple theoretical models that can replicate the coherence in the experimental data. To address this, my research focuses on obtaining precise structural data for key light-harvesting proteins, which will enable a clearer understanding of the role of quantum coherence in light harvesting. The efficient transfer of excitons within cryptophytes involves a blend of coherent and incoherent steps. Coherence is observed in intra-protein interactions, whereas transfer between PBPs appears incoherent. We postulate that the PBP connecting the antenna to the photosystem operates coherently. Recent investigations have unveiled two distinct quaternary structures of PBPs: open and closed forms. Closed forms exhibit coherent beats, while open forms do not. The coherence within individual protein molecules in the cryptophyte antenna is assumed to be vibronic coherence, encompassing electronic and vibrational degrees of freedom. In contrast, energy transfer between proteins is predominantly incoherent, primarily occurring through Förster resonance energy transfer (FRET). The main goal of my research project is to determine the role of quantum coherence in the light harvesting systems of cryptophytes. Specifically, we are investigating the quantum mechanical nature of the linking protein that connects the antenna proteins with the photosystem in cryptophytes. To achieve this objective, we employ a combination of experimental and computational methods. First, we are determining 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 are building 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.Understanding the quantum biological nature of the linking protein holds immense promise for 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.

Keywords: Light-Harvesting, Cryptophytes, Cryo-EM, Molecular Dynamics Simulations, Quantum Calculations

HFSP Reference Number:
HFSP Award Category: Awardee HFSP Cross-Disciplinary Fellowship
HFSP Award Year: 2024

SEQUENCING CARBOHYDRATE CHAINS ONE-AT-A-TIME

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Polysaccharides are the most abundant biopolymers on Earth and play essential roles in all living systems. As with all biomolecules, the empirical rule that 'function follows form' is particularly relevant to polysaccharides, where structural modifications can profoundly influence their functionality. However, determining and fully understanding their structures—and how these structures govern bioactivity—remains a significant challenge due to their complexity and heterogeneity. Electrospray Ion Beam Deposition (ESIBD) offers a novel approach to addressing this challenge by transferring polysaccharides from solution onto a surface in vacuum, enabling their individual imaging via Scanning Tunnelling Microscopy (STM). This combined ESIBD+STM methodology provides unprecedented single monosaccharide resolution, facilitating the structural elucidation of the polysaccharides one-chain-at-a-time. Here, we present the preliminary application of this technology to sequence the structures of two classes of linear polysaccharides: (i) chitopolymers and (ii) glycosaminoglycans (GAGs). GAGs are sulfated polysaccharides which play key roles in growth factor interactions, cell recognition, and signalling, while chitopolymers are acetylated polysaccharides which are crucial in biomaterials, immune signalling, and drug delivery biotechnology. Using ESIBD+STM, we highlight these sugar modifications and pave the way for deeper insights into the primary structure of these complex molecules.

Keywords: Scanning Tunnelling Microscopy (STM), Electrospray Ion Beam Deposition (ESIBD), Single-molecule imaging, Polysaccharide structure, Glycosaminoglycans and chitopolymers

HFSP Reference Number: RGEC31/2023
HFSP Award Category: Awardee Research Grant Early Career
HFSP Award Year: 2023

UNRAVELING ORGAN SIZE DETERMINANTS USING LIMB-SPECIFIC INTER-SPECIES CHIMERAS

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As a developmental biologist, I am interested in how combination and coordination of cell behaviours leads to organ-level outcomes, and how these developmental processes changed during evolution to give rise to diversity of organ form and function. For example, why limbs of different species grow at different speeds and/or for different times? The variations of limb size and proportions indeed provide a powerful model to study evolutionary diversity. Classic inter-species transplantation experiments suggested that limb growth is autonomous, as growth parameters were unaffected by the host. However, we posit these experiments were flawed because they were performed after the fate of the limb cells was irreversibly determined. In fact, recent studies suggest that signals from the body influence limb size. To shed light into this field, we would ideally study and compare organisms at key positions of the tree of life in vivo. However, this approach would not easily distinguish how intrinsic factors (genetic programs) interact with extrinsic factors (cellular microenvironment). I thus aimed to use reductionist approaches to define the intrinsic and extrinsic mechanisms controlling limb size and proportions, by leveraging the use of species with very different limb growth parameters. My project is divided into two major parts, in vivo and in vitro chimeras. For the former, we use host mouse embryos genetically incapable of forming the limbs. When these embryos are injected with exogenous pluripotent stem cells, the limbs are derived from these cells. We are using cells from jerboas (Jaculus jaculus), because of their disproportionally long feet, despite having a comparable body size to mice. While timed mating is well established in mice and allows to precisely obtain blastocysts to derive embryonic stem cell lines, this is not possible with jerboas. This is also the cause for the scarce data on their embryonic development, as female pregnancy only becomes obvious once most of gestation has taken place. As a substitute for embryonic stem cells, we created jerboa induced pluripotent stem cell lines (jiPSCs) from fetal fibroblasts, by providing them exogenous pluripotency and self-renewal related genes. The in vitro chimeras approach relies on the formation of artificial limbs (synthepods) in which cells of different species are mixed in 1:9 ratios to analyse local extrinsic effects of the abundant cell type over the minority's genetic program. By using these different sets of experiments, we expect to shed new light into the classic question of organ size determination. In my presentation, I will talk about my project and how every step has the potential to open up new avenues in the culture and modification of pluripotent stem cells from non-model organisms, and to improve the generation of chimeras between distant species, which will be a major breakthrough in evo-devo.

Keywords: Limb Development, Jerboa, Ipscs, Chimera, Organoid

HFSP Reference Number: LT0020/2023-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

ENGINEERING NEXT-GENERATION PROXIMITY LABELING ENZYMES FOR SPATIAL PROTEOMICS IN LIVING CELLS

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ABSTRACT
Molecular interactions and spatial compartmentation underlie all of cell biology, and are frequently disrupted in disease. Traditional methods for elucidating these interactomes and organelle proteomes, such as by biochemical fractionation, are plagued by false positives and false negatives. Proximity labeling (PL) has emerged as a cornerstone technique, enabling capture of spatial proteomic information in living cells through the use of engineered promiscuous enzymes targeted to specific macromolecular complexes or organelles of interest. While TurboID and APEX2, developed by our laboratory, have been widely adopted, they are limited for (1) high-resolution interactome discovery in vivo and (2) cell surface proteome mapping in vivo.
I will present work on two state-of-the-art enzymes, FlexID and LaccID, developed to meet these specific challenges. FlexID, engineered through directed evolution and computational design from bacterial lipoic acid ligase, uses diverse non-biotin small molecule substrates to promiscuously tag only proteins in direct contact, providing unprecedented spatial resolution and labeling under mild, in vivo-compatible conditions. LaccID, engineered from a fungal multicopper oxidase, has selective cell surface activity, uses a diverse panel of non-biotin substrates, and also catalyzes labeling under mild, in vivo-compatible conditions. I will describe the engineering, characterization, and preliminary application of these next-generation proximity labeling technologies to problems in the immuno-oncology field.

Keywords: Protein engineering, proximity labeling, spatial proteomics

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HFSP Award Category: Awardee Long-Term Fellowship
HFSP Award Year: 2025

PHOTOSYNTHETIC ENERGY TRANSFER: MISSING IN ACTION (-DETECTED SPECTROSCOPY)?

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Most life depends on solar energy, which plants capture and convert through photosynthesis. The complexity of nature is already revealed at the molecular level, where the photosynthetic protein scaffold controls the orientation, spacing and local environment between pigments. The result is a carefully tuned arrangement of coupled pigments, producing delocalized excited states, i.e., excitons. The protein–pigment complexes form larger networks which act as an “energetic funnel” resulting in ultrafast exciton propagation to the reaction center where the initial steps of energy conversion occur. Probing exciton dynamics is challenging since the typical processes span a vast range of timescales: from femtoseconds to nanoseconds. In order to understand the design principles of photosynthesis it is necessary to probe the energy transfer on the ultrafast timescale. Ultrafast dynamics can be studied by pump-probe (PP) spectroscopy, which uses one ultrashort pump and one ultrashort probe pulse and measures the pump induced transient change in absorption over the interpulse delay T. PP spectroscopy, although widely used to probe lifetimes, cannot distinguish the contributions of spectrally close transitions since the excitation frequency is not resolved. In coherently detected two-dimensional (2D) spectroscopy two pump pulses with another delay are used. Fourier transformation over this delay obtains the excitation frequency axis while the detection frequency axis is measured via a spectrometer. The 2D map correlates electronic transitions in a manner that is similar to how 2D NMR spectra correlates nuclear spin transitions. Energy transfer can be followed by the dynamics of the off-diagonal peaks in 2D maps, directly reflecting transfer from one subsystem to another.
In recent years, a new variant of ultrafast 2D spectroscopy emerged utilizing fluorescence as a signal. In addition to fluorescence, other observables such as photocurrent, electrons, and ions can also be used for detection. This so-called action-detected 2D spectroscopy offers increased sensitivity and allows, for example, high-resolution 2D spectra in the gas phase, in operando studies of devices, and spatially resolved 2D spectra down to the single-molecule level. While action-detected 2D spectroscopy is rapidly developing, a detailed analysis of exciton transfer signatures in this field remains lacking.
Here, I present an example of action-detected spectroscopy of a multichromophoric system. I will discuss our recent results of fluorescence-detected 2D electronic spectroscopy (F-2DES) of the light-harvesting II (LH2) complex from purple bacteria. We demonstrate that the B800–B850 energy transfer process in LH2 appears with 100% contrast in coherently detected 2D, whereas in F-2DES it is only weakly observable. We explain the weak signatures using a disordered excitonic model including experimental conditions. We derive a general formula for the presence of excited-state signals in multichromophoric aggregates, considering factors such as the aggregate size, geometry, excitonic coupling and disorder. We find that the prominence of excited-state dynamics in action-detected spectroscopy offers a unique probe of excitonic delocalization in multichromophoric systems.

Keywords: Photosynthesis, Time-resolved spectroscopy, Action-detected spectroscopy

HFSP Reference Number: LT0056/2024-C
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HFSP Award Year: 2024

STRUCTURAL PHYLOGENETICS AND THE EVOLUTION OF THE BACTERIAL FLAGELLUM

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Protein structure is more conserved than protein sequence, and therefore may be useful for phylogenetic inference beyond the “twilight zone” where sequence similarity is highly decayed. Until recently, structural phylogenetics was constrained by the lack of solved structures for most proteins, and the reliance on phylogenetic distance methods which made it difficult to treat inference and uncertainty statistically. AlphaFold has mostly overcome the first problem by making structural predictions readily available. We address the second problem by redeploying a structural alphabet recently developed for Foldseek, a highly-efficient deep homology search program. For each residue in a structure, Foldseek identifies a tertiary interaction closest-neighbor residue in the structure, and classifies it into one of twenty “3Di” states. We test the hypothesis that 3Dis can be used as standard phylogenetic characters using a dataset of 53 structures from the ferritin-like superfamily, and a set of 56 mitoCOGs, conserved orthologous proteins thought to be found in the mitochondrion of the Last Eukaryotic Common Ancestor. In both cases, single-protein 3Di-enhanced datasets match reference trees better than traditional amino-acid datasets. We then apply the method to several difficult-to-resolve questions in flagellum evolution, such as the relationship of MotAB to nonflagellar relatives ExbBD/TolQR, ZorAB, and GldLM. These results suggest that structural phylogenetics could soon be routine practice in protein phylogenetics, allowing the re-exploration of many fundamental phylogenetic problems.

Keywords: Structural Phylogenetics, Bacterial Flagellum, Motab, 3Di, Mitocogs

HFSP Reference Number: RGP0060/2021
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HFSP Award Year: 2021

DECODING THE MECHANISMS OF CONDENSATE-MEMBRANE INTERACTIONS IN CHANGING ENVIRONMENTS: THE FOCUS ON NEURONAL SYNAPSES

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Compartmentalization of cellular processes into membrane-bound and membrane-less organelles is vital for cell function, enabling precise regulation of signaling, trafficking, and homeostasis. Recent discoveries have revealed the dynamic interplay between membrane-less organelles—known as biomolecular condensates—and membranes, highlighting their crucial role in coordinating cellular activities such as neurotransmitter release. We investigate the condensate-membrane interaction at the synapse using two condensate-forming proteins: synapsin-1, the most abundant soluble neuronal protein essential for clustering of synaptic vesicles, and PDZD8, an ER-resident proteins critical for the formation of membrane contacts between ER and mitochondria. Our data indicate that lipid composition determines the level of interaction between both synapsin-1 and PDZD8 condensates with membranes. Moreover, we established 2D graphene sensors for characterizing electric properties of these condensates and discovered that condensates accumulate electric potential at their interfaces, suggesting the biomolecular condensates as the putative mesoscale capacitors. In fact, the acute disruption of cytosolic osmolarity led to the disruption of condensates morphology, indicating the dependance of condensates from local ion concentration. Nowhere is this dependance more relevant than in the context of neuronal synapses where activity-dependent depolarization leads to transient ion fluxes. Our cellular models and electrical sensors will help dissect how the changing environmental conditions impact the condensate-membrane signaling cascade.

Keywords : Synapses, Condensates, Membranes, Changing Environments

HFSP Reference Number: RGEC32/2023
HFSP Award Category: Research Grant Early Career
HFSP Award Year: 2023

ARTIFICIAL ALLOSTERIC PROTEIN BIOSENSORS FOR HEART FAILURE DETECTION AT POINT-OF-CARE

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Heart failure has a 50% mortality rate within 5 years of diagnosis, and currently, 25% of heart failure patients below 65 years of age are re-hospitalised within 30 days of being discharged. Adequate accessibility to decentralised testing methods such as point-of-care diagnostics is key to accurately identifying and stratifying heart failure patients' risks after being discharged, potentially avoiding regular and repeated hospitalisations and/or death.
To address this current unmet need, we developed synthetic recognition elements, miniature antibody-like binding proteins, that bind with high affinity to various heart failure salivary-based biomarkers. We optimised their selection and specificity to the target biomarkers using mRNA display, a high-throughput directed protein evolution technique that allowed us to select recognition elements with either independent or co-operative binding mechanisms.
By integrating these recognition elements into versatile biosensor cores, we developed sensors capable of detecting and quantifying two different heart failure biomarkers in saliva with low nanomolar sensitivity. These biomarkers were validated with saliva samples from heart failure patients, demonstrating the potential of our biosensors in real-world applications.
Using the combination of our in-house mRNA display selection and validated biosensor construction pipeline, we have successfully created a range of heart failure biosensors using only synthetic recognition elements. We have established the foundations of a rapid and integrated pipeline for developing protein-based biosensors, designed to detect a wide range of protein and small molecule targets for use in various research and industry applications.

Keywords: Biosensors, Monobody, Mrna Display,

HFSP Reference Number: RGP0002/2018
HFSP Award Category: Alumni Research Grant Program
HFSP Award Year: 2018

VISUALIZATION OF DIETARY LIPID TRANSPORT THROUGH ENTEROCYTES

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Lipids are essential molecules for energy production, cellular membrane components, and signal transduction. More than 2000 lipid species are present in eukaryotic cells, however, the physiological significance of lipid diversity remains unclear. A large variety of dietary lipids from plants, animals and bacteria are taken up in enterocytes in small intestine. I aim to understand how enterocytes take up, process, and secrete different lipid species through their endomembrane system.
I address this question by combining state-of-the-art fluorescent imaging of bifunctional lipid probes with an organoid culture system. Bifunctional lipid probes contain two minimal modifications, a diazirine group for photo-crosslinking and an alkyne group for click chemistry. By feeding a library of bifunctional lipid probes to small intestinal organoids, lipid transport can be observed in pulse chase experiments with sub-organelle resolution.
To reconstitute intestinal lipid uptake, I developed a lipid loading method using natural bile acids, which enable solubilization and initial apical uptake of lipids. I found that the efficiency of lipid uptake varied depending on the different bile acids. Tuning bile acid composition enabled me to mimic physiological lipid uptake, which is a key prerequisite to study lipid transport in enterocytes.
Second, I optimized the lipid imaging method for absorptive cells using Caco-2 cell line, which exhibits the characteristics of enterocytes. My preliminary data show that lipid transport from the apical membrane to the endoplasmic reticulum (ER) is significantly faster in enterocytes compared to other cell lines. The fast transport of absorbed lipids to the ER appears to be via the non-vesicular transport system. These results suggest that absorptive cells have specific machineries for efficient lipid metabolism.
In conclusion, a method for visualizing lipid transport in absorptive cells has been established. Absorptive cells might regulate specific lipid transport mechanisms to maximize uptake of dietary lipids. The next steps will be to quantify the dynamics of various lipid species and also to apply these lipid imaging methods optimized in the absorptive cells to small intestinal organoid cells.

Keywords: Lipids, Absorptive Cells, High-Resolution Imaging

HFSP Reference Number: LT0031/2024-L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2024

CHARACTERIZATION OF HYDROGEL MATRIX FOR EVALUATING SCHISTOSOME EGG MIGRATION

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Schistosomiasis, a parasitic disease caused by Schistosoma worms, is highly prevalent in Africa, South America and parts of Asia (1). Schistosoma species have complex life-cycles involving infection of a freshwater snail intermediate host as well as a mammalian definitive host, such as humans (2). Although attempts have been made to clarify the biology of immune-dependent process of egg granuloma formation, which facilitates the parasite egg excretion from the mammalian host (2), how Schistosoma eggs migrate inside the human body and are eventually excreted to continue the complex life cycle remains an outstanding research question. In this study, from a tissue mechanics viewpoint, we hypothesize that contractile forces produced by fibroblasts during granuloma formation may provide propulsion forces to aid the egg migration process. To understand this phenomenon, we are developing an in vitro microphysiological system incorporating microscopic imaging to allow us to track the interaction between the eggs and fibroblasts during granuloma formation. To provide a microphysiological environment for examining the migration process, polyacrylamide gels with optimized stiffness were prepared. Being an important physical characteristic of a gel, stiffness was tuned and characterized using a rheometer to achieve a Young's modulus range of 7.5~12 kPa. To this end, the stiffness of the gels casted using Biorad's fast-cast acrylamide kit (3) was modified by adjusting the volume of distilled water from 0~200 mL while maintaining the concentration of the stacker and resolver. To quantify egg motion on the gels, fluorescence beads were embedded on top of the polyacrylamide gels to provide pseudo spatial markers for indirectly estimating the spatial egg displacement under microscopic imaging. Bead displacement was captured by a camera mounted on an inverted fluorescent microscope. To extract spatial velocity vectors, we used the widely accepted method of particle imaging velocimetry (PIV) (4). For this purpose, we developed a software algorithm in MATLAB that utilize spatial cross-correlation and fast Fourier transform (FFT) to retrieve the local velocity vectors. The algorithm could successfully analyze test images of size 256×256 pixels in just a few seconds, making it possible to track fibroblast-induced egg displacement on the gels based on the captured microscopic images.

REFERENCES:
(1) Hotez, P. J., et al., PLOS Neglected Tropical Diseases, 8, e2865, 2014; https://doi.org/10.1371/journal.pntd.0002865
(2) Schwartz C., and Fallon. P. G., Frontiers in Immunology, 9, 2492, 2018; doi: 10.3389/fimmu.2018.02492
(3) https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10033918.pdf
(4) Adrian, R. J., and Westerweel, J., Particle image velocimetry. No. 30., Cambridge University Press, 2011.

Keywords: Schistosoma Egg Migration, Microphysiological System, Hydrogel Stiffness, Fluorescence Microscopy, Displacement Mapping

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HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

UNRAVELLING THE MECHANISM OF INTESTINAL SCHISTOSOME EGG MIGRATION IN A COMPLEX HOST ENVIRONMENT

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Schistosoma mansoni, the causative agent of intestinal schistosomiasis, poses a significant public health challenge in tropical regions. One of the most enigmatic aspects of S. mansoni infection is the migration of its eggs, which lack intrinsic mobility, through host tissues to the intestinal lumen for excretion. While endothelial cells have been suggested to play a role in facilitating this process, the role of other cell types, such as fibroblasts, remains poorly understood. Furthermore, it remains unclear why some eggs successfully migrate to the gut lumen, while others become trapped in tissues, leading to granuloma formation and contributing to disease pathology. Understanding these mechanisms may offer new strategies for controlling S. mansoni infection and its associated pathologies.
This study aims to unravel the mechanisms underlying egg migration by examining tissue remodeling, immune responses, and egg gene expression during infection. Mice were infected with S. mansoni cercariae and sampled weekly for twelve weeks to assess tissue changes, immune responses, and egg-related gene expression over the course of infection. The study focuses on four main aspects: (i) A multiplex cytokine ELISA to profile key Th1/Th2 cytokines, such as IL-4, IL-10, TNF-α, and IFN-γ, to monitor immune balance and regulatory responses at different stages of infection; (ii) Characterization of T-cell populations using flow cytometry from blood and spleen samples, with markers such as CD4, CD25, FoxP3, and IFN-γ to evaluate how S. mansoni infection modulates immune responses and T-cell differentiation; and (iii) To investigate the remodeling of liver, lung, and spleen tissues around migrating eggs using immunohistochemistry. Specifically staining of markers for fibroblasts (α-SMA), endothelial cells (CD31), and macrophages (CD68) to visualize granuloma formation and cellular interactions. (iv) Analyze gene expression profile of S. mansoni eggs, surrounding host cells, genes involved in matrix degradation (e.g., metalloproteinases) and immune modulation using quantitative PCR (qPCR).
Finally, these in vivo findings will be correlated with data obtained from our in vitro 3D microphysiological system to explore the potential mechanistic insights of egg migration in a controlled environment.

Keywords: Schistosomiasis, Fibroblasts, Cytokines, Granuloma, Gene Expression

HFSP Reference Number: RGP020/2023
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2023

HIJACKING MOSQUITOES: INFECTION DYNAMICS OF DENGUE VIRUS IN THE MOSQUITO BRAIN

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Mosquitoes are considered the deadliest animals on earth due to their potential to transmit devastating pathogens to humans. Aedes aegypti is a highly invasive mosquito species and the primary vector for arboviruses such as dengue and Zika. Arboviruses and mosquitoes share one common target: humans. Transmission of arboviral diseases depends on the success of infected female mosquitoes in obtaining human blood, which they require to produce eggs. Certain vector-borne pathogens are known to manipulate insect host behavior to enhance disease transmission, for instance by making infected vectors more aggressive towards humans. Nevertheless, the extent and mechanisms by which arboviruses affect Aedes aegypti behavior are unknown.
Our hypothesis is that dengue virus infection alters the mosquito brain and sensory circuits to make them more efficient vectors for arboviruses. Preliminary results based on behavioral assays show that dengue infection increases mosquito biting and feeding frequency, which enhances viral transmission. To understand the molecular mechanism leading to these behavioral changes, we fed female mosquitoes with blood containing live and heat-inactivated dengue virus. We combined bulk RNA sequencing of dengue-exposed and unexposed individual mosquito brains and carcasses with high-resolution whole brain immunofluorescence imaging, revealing the dynamics and replication hotspots of dengue infection in the brain and creating a global transcriptomic atlas of infected and uninfected tissues. Our results reveal a sustained neurotropism for dengue virus and expand our understanding of the complex interactions between mosquito vectors and arboviruses.

Keywords: Mosquitoes, Dengue Virus, Vector Biology, Neuroethology

HFSP Reference Number: LT0012-2023L
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

ROBUST CYTOPLASMIC PARTITIONING BY SOLVING AN INTRINSIC CYTOSKELETAL INSTABILITY

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Early development across vertebrates and insects critically relies on robustly reorganizing the cytoplasm of fertilized eggs into individualized cells. This intricate process is orchestrated by large microtubule structures that traverse the embryo, partitioning the cytoplasm into physically distinct and stable compartments. Despite the robustness of embryonic development, here we uncover an intrinsic instability in cytoplasmic partitioning driven by the microtubule cytoskeleton. We reveal that embryos circumvent this instability through two distinct mechanisms: either by matching the cell cycle duration to the time needed for the instability to unfold or by limiting microtubule nucleation. These regulatory mechanisms give rise to two possible strategies to fill the cytoplasm, which we experimentally demonstrate in zebrafish and Drosophila embryos, respectively. In zebrafish embryos, unstable microtubule waves fill the geometry of the entire embryo from the first division. Conversely, in Drosophila embryos, stable microtubule asters resulting from reduced microtubule nucleation gradually fill the cytoplasm throughout multiple divisions. Our results indicate that the temporal control of microtubule dynamics could have driven the evolutionary emergence of species-specific mechanisms for effective cytoplasmic organization. Furthermore, our study unveils a fundamental synergy between physical instabilities and biological clocks, uncovering universal strategies for rapid, robust, and efficient spatial ordering in biological systems.

Keywords: Embryogenesis, Microtubules

HFSP Reference Number: LT000920/2020-C
HFSP Award Category: Alumni Cross Disciplinary Fellowship
HFSP Award Year: 2020

CUTICULAR HYDROCARBON SENSING MEDIATES GROUP FORMATION IN THE CLONAL RAIDER ANT

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The Rockefeller University

The propensity to form groups is a fundamental step in the evolution of animal societies, like those of ants. However, the sensory and neural mechanisms underlying group formation in ants remain largely unknown. An odor cue can be expected to mediate group formation in ants, similarly to other gregarious behaviors in insects, but this sensory cue has not yet been described in any ant species. Here, we provide evidence for a low-volatility odor cue that mediates aggregation/nest formation in the clonal raider ant (Ooceraea biroi). We describe our efforts to pinpoint the chemical basis of this odor cue, showing that cuticular hydrocarbons (CHCs) are important aggregation cues. We further describe our efforts to pinpoint what part of the CHC profile is used in the decision to aggregate. We show that young ants are innately attracted to a specific CHC compound, but as ants grow older, they start using the fuller CHC profile of nestmates, possibly as a result of a learning process. Finally, we describe preliminary results on how these aggregation cues are represented in the antennal lobe, the major chemosensory region of the ant brain, using a genetically encoded calcium indicator. Overall, this study sets the stage to gain a mechanistic understanding of how sensory perception has evolved to sustain group living in ants, with broader implications for understanding the sensory and neural adaptations that enable the evolution of sociality.

Keywords: Sociality, Group Formation, Aggregation Cues

HFSP Reference Number: LT0023/2023
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

LIPOSOMAL NANOSENSORS FOR ULTRASENSITIVE NEUROCHEMICAL DETECTION BY MRI

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Understanding how neurochemicals shape brain activity requires imaging tools that can capture their dynamics with high sensitivity and resolution. However, current molecular-level functional magnetic resonance imaging (molecular fMRI) techniques struggle to detect neurotransmitter activity at physiological levels due to the limited sensitivity of existing paramagnetic sensors. To address this challenge, our lab has developed liposomal nanoparticle reporters (LisNRs)—a new class of highly sensitive molecular imaging probes that generate robust MRI contrast changes at nanomolar concentrations.1 LisNRs achieve this by encapsulating high concentrations of gadolinium-based contrast agents within liposomes while incorporating analyte-responsive molecular components that control membrane permeability. This design allows for amplified MRI signals upon target molecule binding. We first validated this approach with a biotin-responsive LisNR, demonstrating its potential for molecular imaging.2 More recently, we engineered calcium-sensitive LisNRs to monitor Ca^{2+} dynamics, which play a crucial role in neuronal excitability and synaptic transmission. By integrating rationally designed Ca-responsive lipidic water channels into the liposomal membrane, we successfully tracked Ca^{2+} variations in vitro using MRI. We are now working to bring this technology into in vivo applications and expanding our approach to develop LisNRs that respond to specific neurotransmitters. These nanosensors hold the potential to revolutionize functional neuroimaging by providing a highly sensitive, noninvasive way to map neurotransmitter activity with exceptional specificity. This could open new doors for studying brain function, disease mechanisms, and therapeutic interventions.

References:
1. Simon J et al. (2023) Nat Biomed Eng 7: 313–322.
2. Das S et al. In Revision

Keywords: Liposomal Nanosensors, Calcium Sensors, Molecular Fmri, Neurotransmitter Detection, MRI Contrast Agents

HFSP Reference Number: RGP0037/2022
HFSP Award Category: Awardee Cross Disciplinary Fellowship
HFSP Award Year: 2022

CROSS-SPECIES INSIGHTS INTO SKELETAL MUSCLE HOMEOSTASIS

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The commonality between various muscle diseases is the loss of muscle mass, function, and regeneration, which severely restricts mobility and impairs the quality of life. With muscle stem cells (MuSCs) playing a key role in facilitating muscle repair, targeting regulators of muscle regeneration has been shown to be a promising therapeutic approach to repair muscles. However, the underlying molecular mechanisms driving muscle regeneration are complex and poorly understood. Through a genetic screen in Drosophila, we have identified a transcriptional factor, Deformed epidermal autoregulatory factor 1 (Deaf1), as a new regulator of muscle regeneration. We showed that Deaf1 targets to PI3KC3 and Atg16l1 promoter regions and suppresses their expressions, thus inhibiting autophagy. Deaf1 depletion therefore induces autophagy which blocks MuSC survival and differentiation. In contrast, Deaf1 overexpression inactivates autophagy in MuSCs, leading to increases in protein aggregates and cell death. The fact that Deaf1 depletion and overexpression both lead to defects in muscle regeneration highlights the importance of fine tuning of Deaf1-regulated autophagy during myogenesis. Significantly, we further showed that Deaf1 expression is altered in sarcopenic and cachectic MuSCs. Manipulation of Deaf1 expression can attenuate muscle atrophy and restore muscle regeneration in the mouse models of sarcopenia and cancer cachexia. Our findings together unveil a critical role for Deaf1 in muscle regeneration, providing insights into the development of uncovering new therapies against muscle atrophy.

Keywords: Muscle Atrophy, Autophagy

HFSP Reference Number: LT000243/2015
HFSP Award Category: Alumni Long-Term-Fellowship
HFSP Award Year: 2015

FILMING INSECTS IN ACTION USING FAST LOCK-ON TRACKING

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The University of Queensland

Insects like bees play essential roles in nature, agriculture, and even human health, but many aspects of their behaviour remain poorly understood. One reason is that it's incredibly difficult to film small, fast-moving insects clearly, especially outdoors where they naturally behave. To address this challenge, we've developed Fast Lock-On (FLO) tracking, a method that uses a tiny reflective marker attached to the insect and image sensors to detect its position. Motorized mirrors or cameras are then automatically steered to stay locked onto the insect, allowing researchers to capture sharp, detailed video even as it moves quickly and unpredictably. What makes FLO especially useful is its flexibility: the system can work with high-speed cameras to capture rapid movements or be mounted on a drone to follow flying insects like bees over long distances. In our tests, we successfully tracked a flying honey bee using a drone-based FLO system and captured high-resolution videos of bee foraging and locust jumping. By making it possible to record insects in their natural environments with unprecedented clarity, this technology opens the door to deeper insights into how insects navigate, behave, and interact with the world, insights that could benefit ecology, robotics, and more.

Keywords: Insect Tracking, Insect Behaviour, Insect Flight

HFSP Reference Number: LT000221/2019
HFSP Award Category: Alumni Cross Disciplinary Fellowship
HFSP Award Year: 2019

PROBING THE NEURAL CIRCUITS FOR CONSCIOUS AWARENESS

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Conscious perception is a fundamental aspect of human cognition. However, providing a comprehensive empirical account of consciousness remains one of the greatest challenges in modern neuroscience. Theoretical descriptions propose mechanisms, but their realization in neural tissue is scarcely tested. A significant gap in knowledge hindering progress is knowing where precisely to investigate. That is, the identification of which brain areas are essential for conscious perception remains elusive. Are there specific brain areas consistently engaged for conscious awareness, and do any contribute more than others? Our objective is to map the functional connectome for stimuli entering and being maintained in conscious awareness. We hypothesize that there is a core set of brain areas necessary for the conscious awareness of perceivable stimuli. To test this hypothesis, we will employ electromicrostimulation with functional magnetic resonance imaging (EM-fMRI) in behaving monkeys. Animals are implanted with electrode arrays that span many brain areas. By stimulating different brain areas while the animal is in an MRI scanner, we aim to determine which brain areas are consistently active when an animal is consciously aware of a stimulus.

Keywords: Consciousness, Neuroimaging, Neurophysiology, Neurostimulation

HFSP Reference Number: LT0001-2023
HFSP Award Category: Awardee Long-Term-Fellowship
HFSP Award Year: 2023

NEURONAL COMMUNICATIONS THROUGH LIPIDS; ADVANCES IN MASS SPECTROMETRY IMAGING FOR SYNAPTONEUROLIPIDOMICS OF SINGLE CELLS

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Characterising the lipid composition of cells is paramount to better understanding cell biology and disease. For example, neurons and glial cells, such as astrocytes, become dysfunctional with the onset of neurodegenerative diseases. However, the exact metabolic changes that these cells undergo remain largely unknown. One reason may be the presence of cellular sub-populations with distinct lipidomic phenotypes, which are missed through bulk analyses such as chromatography. Recently matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) has been shown to enable high throughput single cell lipidomic studies. However, conventional MALDI can be limited in the lipid classes it can detect from single cells. Here, we have combined a novel plasma post-ionisation technique with MALDI-MSI to enhance lipid coverage and used this to monitor the lipidomic changes occurring throughout the differentiation of induced pluripotent stem cells (iPSC) to neurons. iPSC-derived neurons were cultured on-slide, stained with fluorescent dyes and chemically fixed using 4% PFA. Sample slides underwent brightfield and fluorescence microscopy imaging using a Leica Thunder DMi8 prior to positive polarity MSI at 10 µm pixel sizes using a modified timsTOF Pro equipped with an atmospheric pressure-MALDI and plasma ionisation (SICRIT) source. Microscopy and MSI data were co-registered to allow for demarcation of on- and off-cell areas and segmentation of data. All images showed strong correlation for cell spacing and morphology, with minimal delocalisation of lipids from cells. Single cell mass spectra were dominated by glycerophospholipids such as phosphatidylethanolamine (PE), sphingolipids such as hexosylceramides (HexCer) and ceramides (Cer), and sterols such as cholesterol. Data was acquired across 4 timepoints during a 35 day culture period, and the mass spectral data was used to inform pLSA dimension reduction analyses. This revealed that as stem cells differentiated and matured, multiple unique cell phenotypes were able to be identified through their lipid profiles. In particular, neurons appeared markedly different from glial progenitor cells in their lipid composition(s) and neuronal cell bodies could be distinguished from neuronal processes through differences in their polyunsaturated lipid content. Through further development of our bespoke atmospheric pressure MALDI source (i.e., transmission geometry sample ablation), we were also able to demonstrate MSI at 1 µm pixel sizes. At these spatial resolutions, lipids from discrete subcellular regions were identified within cancer cell lines (i.e., SH-SY5Y and U2OS) and distinguish the Purkinje neurons from surrounding cells in mouse cerebellum. This advance in mass spectrometry imaging begins to pave the path for characterising molecular profiles at discrete locations around the cell, including from subcellular compartments and organelles.

Keywords: Lipids, Mass Spectrometry Imaging, Neurons, Single-cell

HFSP Reference Number: RGP0002/2022-101
HFSP Award Category: Awardee Research Grant Program
HFSP Award Year: 2022

NANOSCALE CURVATURE ENRICHES THE MEMBRANE-ASSOCIATED CONDENSATION OF LAT/GRAB2/SOS1

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[2] Institute of Science and Technology Austria (ISTA)
[3] Yale School of Medicine

Membrane-associated protein condensation plays a pivotal role in diverse biological processes including immune activation, cell adhesion, and synapse formation. However, most of current models assume the condensation initiated on flat membranes, while the actual cell membrane shape alters dynamically in association with various cellular processes like migration, proliferation and cell-cell interaction. Although the interplay between membrane shape and protein condensation is increasingly recognized with evidence showing protein condensation inducing membrane deformation through attractive and repulsive interactions, yet how pre-existing membrane curvature influences protein condensation remains elusive. This raises a critical question about whether membrane geometry can modulate biochemical reactions through protein condensation. Here, we investigate the condensation of the well-studied LAT/SOS1/Grb2 system on curved membrane guided by designed nanostructures in vitro and in live cells. This condensation is known as key players in TCR-related signalling on T cell membrane that contains highly curved microvilli with 50 nm – 350 nm in diameter. Remarkably, the condensation of LAT/SOS1/Grb2 preferentially enriched at curved membrane with 250nm diameter in vitro but not on those with 850nm diameter. Such curvature-guided condensation was found highly dependent on both the conditions of individual molecules (e.g. protein density, membrane mobility) and the intermolecular interactions (e.g. crosslinking strength, cluster diffusion). In live cells, the curvature preference could be effectively modulated among different sites through additional membrane-associated protein assembly, such as actin polymerization. Our findings indicate that nanoscale curvature serves as a critical modulator of the protein condensation on membrane, suggesting membrane geometry regulation as an effective strategy of cells to manipulate spatial signalling transduction.

Keywords: Membrane Curvature, Protein Condensation, Nanopillar, Negative Curvature, T Cell,

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HFSP Award Category: Alumni Research Grant Early Career
HFSP Award Year: 2021

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