23rd HFSP AWARDEES MEETING

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BOOK OF ABSTRACTS

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POSTER SESSION

SPECIAL SESSION

STRUCTURAL DAMAGE TO AXONS RESULTING FROM REPETITIVE MECHANICAL MOTION

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Abstract

The project aims to elucidate how repetitive mechanical motion damages internal structural components, such as microtubules, in axons of neurons and which repair mechanisms contribute to maintaining homeostasis. While traumatic brain injury models have revealed the structural damage caused by large stretching deformations of axons, the effects of subcritical stretching and bending repeated over thousands or millions of cycles have not yet been investigated. In engineering, fatigue and wear limit the lifetime of machines due to accumulating microscopic damage of load-carrying parts. Clearly, cellular mechanisms for self-repair have evolved to support axonal functioning. To identify both the damage mechanisms and the repair mechanisms whose balance maintains the structural and functional integrity of axons in the long term, we are studying Dorsal Root Ganglion neurons which have a typical elongated axon, relatively high tensile strength and are involved in daily repetitive movement. We have developed an experimental setup to exert repeated cycles of stretching and bending deformations on neuronal axons and are further improving it. We are now characterizing the structural damage of axons resulting from repeated deformation cycles as a function of the amplitude and number of deformations using imaging. Our data indicates that the applied strain primarily induces morphological alterations in DRG neurons, influenced by both the intensity and the number of compression/stretching cycles. A 2.5% strain did not induced axon degeneration but did lead to axon buckling. Furthermore, we identified an association between the mechanical strain and post-translational modifications to axonal microtubules. Since microtubules exist as dense bundles in neurons, we studied the effect of external mechanical stress on microtubule bundles and compared with the case of single microtubules. After a single cycle of compression and relaxation, we found that the wave amplitudes of thicker bundles were recovered whereas bundles with a smaller number of microtubules underwent irrecoverable deformation. We also observed that the extent of buckling of microtubule bundles was dependent on the strain rate, although no considerable effect on single microtubules was found. Ultimately, we aim to understand the mechanical aspects of homeostasis in cells, inspire new biomimetic approaches to engineering, and yield a better appreciation of the cell as a “self-repairing machine”.

HFSP reference number: RGP0026/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: HESS, Henry (USA)
Co-Investigators: KAKUGO, Akira (Japan), RAFFA, Vittoria (Italy), SHEFI, Orit (Israel)

WHOLE BODY CONTRACTIONS AS A SOURCE OF POWER FOR THE CIRCULATORY SYSTEM OF THE JELLYFISH AURELIA

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Abstract

All large animals are endowed with vascular systems, which need to be powered to maintain the fluid flow in the vessels. This typically happens via a localized pump, as in the cardiovascular system of mammals and other animals, or by long wavelength peristaltic waves as in the lymphatic system. As a result, invariably, animals experience some degree of pulsatility in their circulation. The dynamics in the waveform that powers it can affect both the development and efficiency of the vascular system. For mammals, pulsatility mostly arises due to the heartbeat, however, as we will discuss, for the primitive jellyfish the source can be quite different: muscle contractions, and the resulting whole body deformations as they swim.

The canal network of the gastrovascular system of Aurelia aurita jellyfish transports nutrients and oxygen to the animal's disk-like body. This planar network consists of several radial canals connecting to a circular canal at the rim of the body, with flow entering through radial canals near the center and spreading to the circular canal at the rim. The flow then returns via branched radial canals to the center before being released into the environment. Flow propulsion mechanisms are thought to involve ciliary action on canal walls and deformation caused by swimming movements, but to this day there are no quantitative studies supporting either hypothesis. In this work we focus on the latter mechanism, propulsion induced by deformation . We produce maps of the subumbrella deformation during swimming, which we use to understand how flow is powered. We develop a mathematical model of ``length-induced'' peristalsis, which maps whole body deformation to fluid propulsion, and use it to predict which swimming mechanisms promote internal flows. Our approach underscores the tight relationship between swimming deformation patterns and efficiency of the digestive system and highlights a new mechanism for flow propulsion in animals. Moreover, given that the jellyfish are evolutionarily very distant to all bilaterians, studying their flow propulsion mechanism helps us better understand the fundamental design principles for animal circulatory systems.

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HFSP reference number: RGP0015/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2023
Principal Investigator: KATIFORI, Eleni (USA)
Co-Investigators: JONES, Elizabeth (Belgium), CORNELISSEN, Annemiek (France)

3D SPATIAL NAVIGATION IN EGYPTIAN FRUIT BATS

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Abstract

Our HFSP project explores mental 3D space-time travel in fission-fusion animal societies. The ability to mentally travel in time, also known as episodic memory, has been crucial in the biological and cultural evolution of humans. It is widely assumed that mental time travel (MTT) abilities separate humans from other animals. Amidst ongoing debates, our project aims to deepen the understanding of MTT by comparing episodic-like memory behaviors of bats, dolphins, and parrots, combined with tests of emerging principles in robotic platforms.

Here we report on a subset of this project that focuses on the flight and lingual echolocation of Egyptian fruit bats, animals equipped with two distal sensing systems, vision and active hearing, for spatial navigation. Our studies investigate the roles of spatial memory, distal sensory cues, and attentional processes in path planning. Using stereo IR-video and microphone arrays, we quantify the flight trajectory, head direction, and echolocation beam aim of bats navigating to a landing perch in the light and the dark.

In spatial memory experiments, bats trained to fly to a perch failed to use sensory cues to land after the perch was displaced by 30 cm in the light and 15 cm in the dark. Follow-up studies suggest that distal cues may influence bat navigation: When the perch and landing locations were rotated by 180 deg, bats navigated to the new location but failed to land. In the 90 deg rotation condition, bats circled around the original location, and in the 135 deg rotation condition, bats showed disorganized flight trajectories. Further studies explore the features of distal landmarks that guide bat navigation.

Another experiment investigates the contributions of covert and overt attention to spatial navigation. Bats are trained to locate a landing perch in the dark and learn the association between a distinct acoustic cue and the physical location of the perch. We compare bat flight trajectories, sonar beam direction, and sonar click rate between valid trials containing reliable cues that accurately signal the physical location of a perch and invalid trials containing cues that misdirect the animal to a location without a perch. The bat's echolocation behavior and flight time to the perch on valid and invalid cue trials serve as metrics of overt and covert attention, respectively. Collectively, these experiments aim to shed light on bat navigation, path planning and the broader understanding of spatial cognition in animals.

Web: https://batlab.johnshopkins.edu/

HFSP reference number: RGP0045/2022
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022
Principal Investigator: WAHLBERG, Magnus (Denmark)
Co-Investigators: MOSS, Cynthia (USA), PEREMENS, Herbert (Belgium), VON BAYERN, Auguste (Germany)

THE ATMOSPHERE: A LIVING, BREATHING ECOSYSTEM?

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Abstract

The atmosphere is the largest potential ecosystem on Earth yet the least understood. Life is found across Earth's three major systems, the lithosphere (land), hydrosphere (water), and atmosphere (air). Yet several factors make life poorly understood in the atmosphere: its low microbially-dominated biomass, sampling methodological challenges, and low awareness of its importance and complexity as an ecosystem. Classically, atmospheric-dwelling microbes are thought to be passively dispersed, without performing metabolic activities or mediating ecological interactions [1]. However, recent work suggests that the atmosphere may be a true ecosystem: containing active resident and transient microbes that profoundly influence biology, chemistry, and climate globally [2,3]. Atmospheric microorganisms influence biodiversity, disease, and potentially climate. Microbes are continually exchanged between land and water through the atmosphere. Their dispersal is one of four key processes controlling community ecology, together with selection, drift, and speciation. Other ecosystem roles of atmospheric microbes are underexplored, though they are implicated as key ice nucleators responsible for cloud formation and precipitation, and are likely sensitive to anthropogenic activities. We lack a global picture of the atmospheric microbiome and whether it is comprised of resident or merely transient microbes. In the last decade, studies have attempted to profile atmospheric communities using molecular methods. These studies suggest that diverse microbiota are present in air, at ~ 10^4 to 10^5 cells/m^3. Atmospheric communities vary across space and time, suggesting some biogeographic structure. However, it is unclear if deterministic or stochastic processes control their assembly. This project will test whether the atmosphere is indeed 'a living, breathing ecosystem.' Our study addresses these knowledge gaps by resolving the composition, capabilities, and activities of atmosphericmicrobes at a global scale. The overarching goal is to distinguish whether the atmosphere is simply a passive dispersal medium for microorganisms, or if they exhibit structure and activity characteristic of a true ecosystem.

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HFSP reference number: RGY0058/2022
HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2022
Principal Investigator: GOORDIAL, Jacqueline (Cananda)
Co-Investigators: BRADLEY, James (UK), GREENING, Chris (Australia), TREMBATH-REICHERT, Elizabeth (USA)

ORAL SESSIONS

PREVENTING ABERRANT REPLICATION IN CANCER CELLS

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Abstract

Loss of genetic control of DNA replication is a hallmark of cancer. Pathways that modulate DNA synthesis can provide good targets for synthetic lethality approaches that specifically target cancer cells, but DNA replication problems that go undetected and are not repaired can hamper genomic integrity. To characterize replication dynamics in cancer cells, we use whole-genome sequencing combined with imaging-based single-fiber analyses to characterize the effects of specific proteins on genome duplication patterns.

Our recent studies inquired how highly aggressive cancer cells, which can tolerate widespread DNA damage, respond to DNA breaks . We found that DNA breakage induce a local, hitherto unreported genome maintenance mechanism that inhibits replication initiation in topologically associating chromatin domains that contain DNA breaks without affecting DNA synthesis at other genomic locations. This pathway is mediated, in normal and cancer cells, by two components of the DNA replication machinery (TIMELESS and TIPIN) and the WEE1 kinase, a cell cycle regulator that actively dislodges TIMELESS and TIPIN from chromatin and prevents DNA synthesis within chromosomal domains that are adjacent to DNA breaks.

In parallel, we have identified a regulatory interaction that selectively starts replication at distinct genomic sites (replication origins). During unperturbed growth, only a fraction of potential replication origins (baseline origins), but not other sites (dormant origins) initiate replication. The mechanism underlying this selective replication origin dormancy is elusive, since both baseline and dormant origins were known to interact with similar protein complexes (pre-replication complexes). We have now identified a protein (MDM2 Binding Protein, MTBP) that selectively associates with baseline, but not with dormant, origins. MTBP dissociates from baseline origins if cells encounter obstacles during DNA synthesis. Another protein, RecQL4, binds dormant replication origins and facilitates the removal of MTBP from chromatin when replication slows or stalls. We will discuss how the Timeless-TIPIN-Wee1 pathway and the MTBP-RecQL4 interaction allow cells to adapt to and recover from replication stress. These observations reveal previously undiscovered vulnerabilities in the DNA replication machinery that may be exploited to therapeutically target cancer cells.

HFSP reference number: LT00489/1993-M
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 1993
Host supervisor: WAHL, Geoff

OPTIMAL MECHANICAL DESIGN OF BIOLOGICAL "NEEDLES": FROM RADIOLARA SPINES TO WHALE TUSKS

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Abstract

Biological needles are ubiquitous puncture devices that serve the purpose of piercing through tissue. From anchoring to a safe home, injecting poison, hunting, or defense against predators, these piercing devices must obey the same physical laws. Two opposing demands constrain needle shape: they must be sharp, to puncture with minimum force, but also rigid, to avoid excessive bending and buckling. Using a minimalist mechanical model, we show that the geometry that best combines these needs is a square root tapering of the circular cross-section. This prediction is in agreement with empirical data on needle morphology (Evans, 2021), and mechanical tests on biomimetic needles (Quan, 2024). Because the piercing force grows with needle diameter for small needles, but with cross-sectional area for larger needles, we further predict that the base diameter of the needle should scale with needle length with a power-law exponent of ¾ for needles below about 1 cm diameter, but of 1 for larger needles. Comparison with empirical data (Evans, 2021; Quan, 2024; Jensen, 2020) across 6 orders of magnitude, from radiolaria spines to whale tusks, confirms these simple predictions, and so suggests that mechanical constraints likely play an important role in shaping the needle morphology.

HFSP reference number: RGY0073/2020
HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2020

SCIENCE OVER THE ABYSS: KNITTING BRIDGES WITH BUTTERFLIES

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Abstract

Science is built on the pursuit of answers to fundamental questions and the constant expansion of our understanding of the world around us. However, this effort has not been without challenges and inequalities. This article critically examines the issue of diversity in science and the notable disparities that persist in global scientific knowledge. Throughout history, the contributions of scientists from diverse regions and cultures have been pivotal to scientific advancement. Nevertheless, significant gaps in terms of access, funding, and recognition in the global scientific community still endure. We use the concept of the "abyss," as a metaphor for the disparities in scientific practices across diverse regions of the world within the context of globalization. We seek to shed light on how the abyss influences the very essence of scientific inquiry, ranging from disparities in access to knowledge to the limitations imposed by technology and resources. This work addresses how socioeconomic, gender, and geographical disparities impact who has the opportunity to engage in and lead scientific research. The decolonization of science and the incorporation of indigenous and local perspectives in research are highlighted as crucial ways to address these disparities. Additionally, the concept of participative science is explored as an inclusive approach that allows diverse communities to take part in scientific research. Ultimately, this exploration of diversity in science and disparities in scientific knowledge seeks to inspire deeper reflection on how we can work together to ensure that science becomes a truly global and representative endeavor, enriched by a multitude of perspectives and the collaboration of people from all corners of the world.

Web: https://orcid.org/0000-0002-1954-1806, https://youtu.be/4C2j-8bsSzU?si=83wKd7rI33rPD6ak

HFSP reference number: RGP0029/2022
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022
Principal Investigator: MONTGOMERY, Stephen (UK)
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THE STRUCTURE OF THE TAD PILUS ALIGNMENT COMPLEX DEMONSTRATES THAT IT FORMS A TRANS-PERIPLASMIC CONDUIT FOR PILUS EXTENSION

Sasha Evans[1]; Iryna Peretiazhko[1]; Sahil Karnani[2]; Jamie Wheeler[3]; Boo Shan Tseng[4]; William Durham[3]; John WHITNEY[5]; Julien Bergeron*[1]

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Abstract

The Tight adherence (Tad) pilus is a filamentous appendage present at the surface of many bacterial species, and plays a pivotal role in the formation of antibiotic-resistant biofilms, presenting a promising target for antimicrobial development. *Pseudomonas aeruginosa,* a human pathogen responsible for most hospital-acquired infections, harbors a Tad system. The Tad pilus consists of a long, external filament that protrudes from the cell surface, and anchored to the cell membranes via its assembly apparatus. Despite its significance, the structure of Tad remains mostly uncharacterized.

We have previously determined the structures of the periplasmic Tad component RcpC, which reveals that it forms a pore through the peptidoglycan layer, through which the pilus filament extends. Our aim is to determine if this forms a continuous complex with the outer-membrane secretin pore, and to determine the structure of the corresponding complex.

To this end, we observed that RcpC co-purifies with the secretin RcpA, demonstrating that they indeed interact. Size-exclusion chromatography with multi-angle light scattering and negative stain electron microscopy (EM) analysis confirmed the formation of a stable oligomeric complex, for which a dataset of cryo-EM was collected. This allowed us to determine the structure of the corresponding complex, however no density was observed for RspA, suggesting a flexible interaction. Finally, we engineered several mutations in RcpC , and assessed their impact on binding to RcpA.

HFSP reference number: RGY0080/2021
HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2021
Principal Investigator: BERGERON Julien, UK

MECHANISTIC INSIGHTS INTO THE FLUIDITY AND RHEOLOGICAL BEHAVIOR OF EPITHELIAL TISSUES USING BIOPHYSICAL MODELS

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Abstract

In the process of embryonic development, tissues experience significant reshaping to create functional organs. Adult animals also face ongoing mechanical stresses and deformations in their cells and tissues to maintain physiological functions. The ability of cells to resist these mechanical forces, as well as to flow collectively, is crucial for both embryonic development and adult physiology. These mechanical changes can be self-generated at the cellular level or imposed externally by adjacent tissues and organs. Past research in tissue mechanics has often focused either on how tissues respond to external forces or on the internal stresses generated within the cells. In contrast, our study integrates both aspects using a 2D active vertex model of confluent tissue. We investigate how external forces applied across the tissue interact with internal stresses arising from cellular movements.

Specifically, we explore how the balance between external and internal forces influences the overall mechanical behavior of the tissue. Our focus is on tissues that are near a transition point between behaving like a solid or a fluid, known as the jamming/unjamming transition. In such tissues, we identify a range of intriguing rheological properties, including yielding, shear thinning, continuous shear thickening (CST), and discontinuous shear thickening (DST). Our model offers a comprehensive framework for understanding the complex, nonlinear rheological behaviors observed in living tissues.

Web: www.dapengbi.com

HFSP reference number: RGP0007/2022
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022
Principal Investigator: Das, Tamal (India)
Co-Investigators: Bi, Dapeng (USA), Serwane, Friedhelm (Germany)

ELECTROSTATIC ATLAS OF NONCOVALENT INTERACTIONS BUILT IN METAL-ORGANIC FRAMEWORKS

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Abstract

Noncovalent interactions form the basis of the organization of matter generally and living systems in particular. We are especially interested in a quantitative analysis of the non-covalent interactions that provide the unique environment found at the active sites of enzymes and are responsible for catalytic function. Compared with covalent chemistry, which is very well developed, non-covalent interactions are much less well characterized. We have devised a unique platform strategy to systematically build noncovalent interactions with arbitrarily chosen chemical groups into precisely designed configurations by using metal-organic frameworks (MOFs) as the molecular scaffold. This design brings a range of biologically relevant chemical functionalities into close and well-defined proximity (distance and orientation), without a direct covalent connection. The magnitude of non-covalent interactions can then be interrogated by using the vibrational Stark effect (VSE). VSE uses the vibrational frequency shifts observed using IR or Raman spectroscopy of selective probes, nitriles (-C$^{\circ}$N) and carbonyls (-C=O) in particular, to extract the electric field or electrostatic interaction associated with the non-covalent interaction. These interactions are created by the charges and dipoles participating in the interactions. Using vibrational spectroscopy benchmarked against computer models, we found the electric field to emerge as a unifying metric for quantifying diverse noncovalent interactions with a common unit, spanning a wide range of more than 100 MV/cm. By synthetically making and spectroscopically testing a collection of noncovalent interactions, we identified unique destabilizing forces as strong as +24 MV/cm in contrast to common interactions that typically stabilize the involved chemical groups. This novel approach provides direct information on non-covalent interactions that can be compared with computational methods which are the basis of widely used simulations of biological systems. We will present examples from enzymes for comparison with these MOF-based frameworks to highlight the importance of this approach.

Web: https://wuttkescience.com/stefan-wuttke/, https://www.boxerlab.stanford.edu/

HFSP reference number: RGP0047/2022 WUTTKE
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022

ADVENTURES IN PROTEIN-PROTEIN AND PROTEIN-MEMBRANE BIOPHYSICS - HIGHLIGHTS FROM BUCK LAB. OVER THE LAST 20 YRS

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Abstract

This presentation provides an overview of the work of the Buck lab. at Case Western Reserve University over the last 20+ years on studies of protein-protein/protein-membrane interaction biophysics. Our principle tools have been solution NMR and molecular dynamics simulations (recently Alphafold and other molecular modeling). The principal systems studied are the plexin and eph receptor transmembrane receptors which play a role in regulating and directing cell migration processes in development and disease. In terms of diseases, the receptors have been investigated in the context of cancer metastasis, pathological angiogenesis and recently in Alzheimer's disease. Apart from elucidating the sequence-structure-function relationships in these receptors and their protein binding partners, our contributions are also to the refinement and extention of concepts in protein dynamics and allostery. As a related project, we examined the transient interactions of the G-domain of K-Ras, and of the bound Raf effector protein, with different membranes.

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HFSP reference number: LTF/1994
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 1994
Fellow: BUCK, Matthias
Host supervisor: KARPLUS, Martin

HIDDEN COMET-TAILS OF MARINE SNOW IMPEDE OCEAN-BASED CARBON SEQUESTRATION

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Abstract

Global carbon-cycle on our planet ties together the living and the non-living world, coupling ecosystem function to our climate. Gravity driven downward flux of carbon in our oceans in the form of *marine snow*, commonly referred to as biological pump directly regulates our climate. Multi- scale nature of this phenomena, biological complexity of the marine snow particles and lack of direct observations of sedimentation fundamentally limits a mechanistic understanding of this downward flux. The absence of a physics based understanding of sedimentation of these multi-phase particles in a spatially and temporally heterogeneous ocean adds significant uncertainty in our carbon flux predictions. Using a newly invented scale-free vertical tracking microscopy, we measure for the first time, the microscopic sedimentation and detailed fluid-structure dynamics of marine snow aggregates in field settings. The microscopically resolved in-situ PIV of large number of field-collected marine snow reveals a comet tail like flow morphology that is universal across a range of hydrodynamic fingerprints. Based on this dataset, we construct a reduced order model of Stokesian sedimentation and viscoelastic distortions of mucus to understand the sinking speeds and tail lengths of marine snow dressed in mucus. We find that the presence of these mucus-tails doubles the mean residence time of marine snow in the upper ocean, reducing overall carbon sequestration due to microbial remineralization. We set forth a theoretical framework within which to understand marine snow sinking flux, paving the way towards a predictive understanding of this crucial transport phenomena in the open ocean.

Web: https://doi.org/10.48550/arXiv.2310.01982

HFSP reference number: LT000704/2021-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2021
Fellow: CHAJWA, Rahul
Host supervisor: PRAKASH, Manu

LIGHT REGULATES IMMUNITY VIA A DIRECT RETINA-TO-LYMPH NODE PATHWAY

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Abstract

Light is a cosmological constant and governs many aspects of life on earth. Complex organisms, including mammals, evolved photoreceptors that perceive environmental light and neurons in their brains that use photic information to optimize physiological responses through efferent neural outputs. Peripheral organs, including immune organs, are richly innervated and receive light-induced brain inputs, which in turn drive rhythmic behaviours, suggesting the presence of a direct brain-to-target pathway conveying photic information. However, whether light can directly modulate immune organs, such as lymph nodes (LNs) remains unknown. LNs are richly innervated structures that act as critical sieves to allow the generation of adaptive immune responses. Here, we demonstrate that light directly influences LN activity via a descending neuronal pathway that connects the retina to LN-innervating sympathetic fibres. Using a combination of state-of-art chemogenetic, optogenetic and pharmacological approaches, we show that light induces activation of a specific class of photoreceptors in the retina, which in turn trigger a descending pathway originating from the locus coeruleus (LC) in the brainstem, which activates sympathetic fibres directly innervating LNs. Additionally, retrograde multi-synaptic tracing of the circuit confirmed the connection between LNs and the LC, further corroborating our findings. Finally, scRNA-seq analysis of LNs allowed for the identification of molecular mechanisms associated with light stimulation. Together, these data provide first evidence for a direct retina-to-LN neuronal pathway that modulates LN physiology, ultimately regulating immune functions.

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HFSP reference number: LT0051/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: DE VIRGILIIS, Francesco
Host supervisor: SCHEIERMANN, Christoph

OPTIMIZING BASE EDITING FOR HIGH-THROUGHPUT ENHANCER MUTAGENESIS IN VIVO

Julia Falo Sanjuan*[1]; Yuliana Diaz-Tirado[1]; Julian Davis[1]; Claudia Medrano[1]; Meghan Turner[1]; Hernan Garcia[1]; Michael Eisen[1]

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Abstract

Understanding the rules that govern gene expression remains a major unsolved challenge in biology. Regulatory sequences, termed enhancers, are composed of binding sites for transcription factors, whose number, affinity, spacing and orientation can play a role in enhancer function. Although the roles of some of these factors are well established, the rules by which binding site arrangement dictate gene expression programs remains poorly understood. One limitation when studying enhancer function in living organisms is the need to create multiple transgenic lines harboring enhancers with the desired mutations. To circumvent this, we are generating tools to create a high degree of enhancer sequence variability in vivo using base editing, and optimizing assays to test the activity of all these variants in a high throughput manner, using STARR sequencing in transgenic S2 cells and in Drosophila embryos by single cell RNA sequencing. As proof of concept, we show the base editor evoCDA1-AID fused to nCas9 can introduce mutations efficiently in cell culture and in vivo, using arrays of 20+ gRNAs targeting GFP. Surprisingly, longer gRNAs increase mutation efficiency and expand the mutating window, which can allow the introduction of mutations in previously untargetable sequences. I will present our progress to introduce mutations in an enhancer in fly embryos and plans to test the functional outcome of these mutations.

HFSP reference number: LT000310/2021
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: FALO-SANJUAN, JULIA
Host supervisor: EISEN, Michael

THE AMINO ACID ALPHABET VERY LIKELY PASSED THROUGH AN ACIDIC INTERMDIATE

Mikhail Makarov[1]; Robin Kryštůfek[2]; Soumya Dutta[3]; Abhishek Singharoy[3]; Stephen Fried*[4]; Klara Hlouchova[1]
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Abstract

Modern proteins are remarkable functional polymers that use a 20-letter amino acid alphabet arranged into specific sequences that arose from billions of years of evolution. However, in the prebiotic stages of Earth's history, some amino acids were not abundant – including all the canonical basic amino acids (lysine, arginine, and histidine) – whereas the canonical acidic amino acids, aspartic acid (Asp) and glutamic acid (Glu), were quite abundant. These findings have stimulated the theory that early peptides may have been highly acidic. On the other hand, it is hard to rule out the possibility that noncanonical basic amino acids served as intermediates that were later displaced as the alphabet evolved. Here, we synthesise and extensively 20haracterize the solubility and folding properties of random peptide libraries "written" with nine amino acid alphabets. The data show that a prebiotically-plausible acidic alphabet featuring Asp and Glu is singular in its capacity to promote peptide folding, and show unambiguously that any inclusion of basic amino acid residues – both canonical and prebiotic, either in isolation or alongside acidic residues – precludes efficient folding. Combined with a large language model for structure prediction, our results moreover show an unusual propensity for this acidic alphabet to promote globular compact structures with high α-helical content even in a random unevolved sequence space. These features are not apparent even for the canonical amino acid alphabet. Altogether, this study has moved us to say that it was very likely the earliest polypeptides on Earth were highly acidic, which served as a key intermediate to produce rudimentary foldable polymers prior to extensive purifying selection.

HFSP reference number: RGEC27/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2023
Principal Investigator: FUJISHIMA, Kosuke (Japan)
Co-Investigators: FRIED, Stephen (U.S.A.), HLOUCHOVÁ, Klára (Czech Republiv), FREELAND, Stephen (U.S.A.)

MODELLING CORTICO-LIMBIC SYSTEM IN VITRO TO STUDY HUMAN NEURODEVELOPMENT AND DISEASE

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Abstract

The limbic system has an essential role in emotion processing and memory formation and encompasses multiple brain regions, including the hippocampus and amygdala. Alterations in these brain structures and their interaction with the cerebral cortex have been highlighted as hallmarks of neurological disorders. However, the neuroanatomy and functionality of the cortico-limbic axis remain largely unknown. Stem cell-derived 3D organoids provide an excellent opportunity to expand our understanding of these complex cellular networks and how their alterations result in disease. Here, we describe novel methods to generate 3D hippocampus and amygdala models and to connect them to cortical organoids, providing a platform to study cortico-limbic system interactions and investigate the impact of neurological disorders on these regions. The cerebral cortex, hippocampus, and amygdala develop from the embryonic dorsal telencephalon in a process that is finely regulated by morphogens. By exposing iPSC aggregates to BMP-WNT or FGF-SFRP signalling and adapting the cultures to growth in spinner-flask bioreactors, we achieved the long-term development of an extensive compendium of hippocampal and amygdala cell types. ScRNA-seq and immunohistochemistry analysis at 9, 18, 30, 90, and 180 days revealed that while at early stages of *in vitro* development, organoids have the potential to generate multiple regions of the developing nervous system, in long-term cultures they show a specific hippocampus and amygdala identity. We investigated the functional activity of our models using a high-resolution multielectrode array system and detected 3D neural network activity at 90 and 180 days in both models. To recreate the interactions between the limbic system and the cerebral cortex, we used previously characterised cortical organoids and our novel hippocampus and amygdala models. We investigated the connectivity among distinct brain regions with long-term live-imaging and functional assays by generating each organoid using iPSC lines engineered to express different fluorescent reporter genes. These newly established models provide an unprecedented platform to investigate how the cortico-limbic system is built and how abnormalities cause disease in order to identify the pathophysiological mechanisms of neurological disorders.

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HFSP reference number: LT0024/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: GARONE, Maria Giovanna
Host supervisor: VELASCO, Silvia

GEARS: A TOOLKIT FOR VISUALIZING AND MANIPULATING ENDOGENOUS PROTEIN FUNCTION

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Abstract

To probe endogenous protein localization and function *in vivo,* we have developed GEARs: ***G****enetically* ***E****ncoded* ***A****ffinity* ***R****eagents*; a collection of short epitope-based tags and their cognate GEAR binders, which can 1) measure protein localization and abundance and 2) perturb protein function *in vivo*. First, after validating the functionality of these reagents using exogenous reporters, we generated GEAR tagged alleles with a novel, rapid CRISPR/Cas9 based protein tagging pipeline in zebrafish. Second, we demonstrate that these tags can be used in conjunction with targeted protein degradation in zebrafish, mice and human cell culture. Next, we used GEARs to examine the native behavior of the pioneer transcription factor Nanog, which is required for genomic reprogramming and transcriptional activation in embryos. Recently, it has been shown that Nanog coordinates the organization of large transcription bodies during the maternal-to-zygotic transition. While live imaging of exogenous, fluorescently tagged Nanog has revealed important molecular behaviors, the physiological relevance of these experiments is unknown. Using a GEAR tagged Nanog, we investigated its endogenous behavior. When visualizing Nanog localization and dynamics by live imaging, we find fewer Nanog clusters that are notably smaller in comparison to exogenously introduced Nanog, further underscoring the physiological relevance of probing endogenous targets. Together, this toolkit provides a versatile system for probing and perturbing endogenous protein function while circumventing challenges associated with conventional gene targeting and is broadly available to the model organism community.

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HFSP reference number: LT-0037/2022-L4
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: Hoppe, Caroline
Host supervisor: Giraldez, Antonio

TOWARDS BIOLOGICAL COMMUNICATION WITH LIGHT

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Abstract

A living neural network communicates with electric signals, providing a communication speed of up to 100 m/s. Similarly, as the shift from electric to optical communication has revolutionized internet communications, we question whether introducing optical communication to a living neural network may provide more efficient and powerful computation at the speed of light.

Within this project, we are working towards realizing a living optically-communicating neural network. The system comprises the transmitting part, the receiving part and the supporting microfluidic part to integrate everything into a single system.

The transmitting part emits fluorescent light based on neuronal activation serving as presynaptic neurons. We optimized the emission so that it is strong enough for the resulting light to be able to excite the receiving neurons. We achieved this by optimizing the optical setup in various ways, including the use of appropriate dichroic mirrors to create an optical cavity.

In the receiving part, we have light-responsive postsynaptic neurons which express an optogenetic actuator sensitive to the emission light of the transmitting part. These optogenetic neurons sense light emission from the presynaptic neurons to evoke postsynaptic action potentials.

The optics and cells are integrated into a microfluidic device, which captures cells in designed area and continuously provides nutrients and initial biological stimulus to the cells. Furthermore, the microfluidic device is designed to be compatible with a multichannel fluorescence microscope. We also automated the fluidic controls with simple graphic interface unit design for reproducible operations.

Overall, this project paves the way to realize optical communication between the cells for the first time.

HFSP reference number: RGY0068/2020
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2020
Principal Investigator: Humar, Matjaž (Slovenia)
Co-Investigators: Choi, Myunghwan (South Korea), Im, Hyungsoon (USA)

FLORAL ASYMMETRY: DISTINGUISHING LEFT FROM RIGHT

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Abstract

Asymmetry is a rare phenomenon in most multicellular organisms, and in plants in particular. In mirror-image flowers, the female and male reproductive organs are asymmetrically positioned, a phenomenon known as enanstiostyly. In dimorphic enantiostyly, individual plants in populations have either left- or right-handed flowers. Our study has investigated the genetic, cellular, evolutionary and ecological basis of this phenomenon in four Wachendorfia species, and the sister genus *Barberetta aurea*. Key questions include what is the genetic basis of enantiostyly, what is the cellular basis by which styles and stamens orientate in opposing directions, how did dimorphic enantiostyly evolve and how are 1:1 morph ratios maintained in populations?

We have shown that the direction of style and stamen displacement is under the genetic control of a hemizygous region, present only in right morph individuals. Two genes, a functional microRNA, miR156 and an auxin biosynthetic gene, YUC-R are conserved in this hemizygous region across the four Wachendorfia species and *B. aurea*. miR156 is exclusively expressed in the styles, and YUC-R, in the opposing stamen of right -morph flowers. Naturally occurring mutants have been identified which lack the *miR156* or YUC-R genes, confirming the functional importance of these genes.

Imaging of *W. paniculata* styles and stamens revealed that the angles of cell files at the base of styles (or stamens) indicate twisting in a left- and right-handed helix, respectively, and this correlates with the deflection of a style (or stamen) to either the left or right. Most populations of *W. paniculata* and *B. aurea* exhibit equal ratios of left- to right-handed individuals. Observations of syrphid flies visiting *B. aurea* flowers indicate that efficient transfer of pollen via the wings of pollinators between left- and right-styled plants may be a selective factor maintaining the asymmetrical deflection of styles in some species.

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HFSP reference number: RGP0036/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: LENHARD, Michael (Germany)
Co-investigators BARRETT, Spencer (Canada), DEINUM, Eva (the Netherlands), ILLING, Nicola (South Africa)

BUZZ-POLLINATION: BEE-FLOWER VIBRATIONAL COUPLING AND ROBO-BUZZER PROTOTYPE DEVELOPMENT

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Abstract

Buzz-pollination, in which a pollinator generates powerful vibrations to rapidly remove pollen from certain types of flowers, is used by more than half of all bee species and over 20,000 species of plants, including crops such as tomato, kiwifruit, and blueberries.

Our project investigates how bee vibrations and bee-anther interactions vary across species, and how these variations impact pollen release. Using field- and lab-collected data on bees' buzzes from around the World, we are developing bee-scale robotic actuators ('robo-buzzers') that will enable us to replicate the mechanical properties of bee vibrations and their coupling with flowers. This will allow us to vary design and operational parameters controllably (which is difficult or impossible to achieve with living bees), as well as to operate in regions of parameter space beyond what bees can achieve naturally, in order to better understand bee behavior and how 'optimal' it is.

Here we present several important aspects of the bee-flower interaction that we have identified through high-speed videos of buzz-pollinating bees, such as amplification of the thorax vibration through the neck and head, and anther-dependent head orientation variation. In particular, we found that, during buzzing, the mandibles of *Bombus terrestris* vibrate at amplitudes up to three times greater than that of their thoraxes, resulting in a doubling of anther motion when the bee is biting (versus not biting) the flower anther (*Solanum dulcamara* and *S. rostratum*). The bees orient their heads so that they are close to perpendicular to the fused anther cones of *S. dulcamara*, but close to parallel or anti-parallel with the more flexible anthers of *S. rostratum*. We hypothesize that this allows the bees to vibrate each anther type at the largest amplitude possible, given the different anther stiffnesses and masses.

These studies have further informed our design of the 'robo-buzzers,' so that they can reproduce the most important features identified from the bee studies, for example, by implementing articulated 'necks' with different properties such as fixed amplification ratios, passive joints, or some combination. The design of the 'robo-buzzers,' together with simplified simulations, has allowed us to hypothesize several possible mechanisms for the observed neck-head vibration amplification, as well as providing insight on mass and stiffness scaling of the bees and flowers.

HFSP reference number: RGP0043/2022
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022
Principal Investigator: VALLEJO-MARIN, Mario (Sweden)
Co-Investigators: JAFFERIS, Noah (USA of America)

BIOADHESIVE ULTRASOUND HYDROGEL INTEGRATED WEARABLE ULTRASOUND TRANSDUCER FOR LONG-TERM NEUROMODULATION

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Abstract

The long-term efficacy of deep brain stimulation (DBS) in brain disorders such as Parkinson's and Alzheimer's disease has been well-established. Additionally, Transcranial Focused Ultrasound (tFUS), one of the emerging DBS technologies, offers a non-invasive approach for precise targeting of deep brain regions with high spatial-temporal resolution. It has demonstrated effectiveness in improving tremors associated with Parkinson's disease.

However, traditional large and heavy ultrasound transducers are not suitable for long-term use in patients. Furthermore, for effective transmission of ultrasound into tissue, ultrasonic gel is required to reduce the air gap between the ultrasound transducer and the skin and match the acoustic impedance. However, commercial ultrasonic gel typically dries within minutes.

In this study, we report a bioadhesive ultrasound hydrogel integrated wearable ultrasound transducer (SFAT-ACFAL) designed to achieve efficient ultrasound intensity for cortical stimulation with a miniaturized size (~ 20 mm in width). We developed a self-focusing acoustic transducer (SFAT) with an air-cavity Fresnel acoustic lens (ACFAL), which demonstrated a 1.5 mm full width at half maximum (FWHM) at a distance of 10 mm. Additionally, utilizing a superabsorbent hydrogel, the bioadhesive ultrasound hydrogel demonstrated remarkably low ultrasound attenuation comparable to commercial gels, high adhesion strength (0.9 N/cm), and long-lasting anti-dehydration properties (7 weeks), indicating its suitability for long-term wearable use.

With the miniaturized transducer and ultrasound hydrogel, our bioadhesive ultrasound hydrogel integrated ultrasound transducer effectively inhibited somatosensory evoked potentials induced by median nerve stimulation via functional electrical stimulation over a period of 4 weeks.

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HFSP reference number: LT0034/2022-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2022
Fellow: Jeong, Jinmo
Host supervisor: Wang, Huiliang

AGING IMPACT ON THE MOUSE PREFRONTAL CIRCUIT

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Abstract

Aging significantly affects executive function, yet its precise impact on circuit-level processes remains elusive. In our investigation, we aimed to elucidate these dynamics by employing calcium imaging and optogenetic manipulation during memory-guided tasks. We observed a gradual decline in working memory maintenance within the mouse medial prefrontal cortex (mPFC) as individuals age.

Moreover, our analysis of resting-state functional connectivity revealed a significant decrease in specific connectivity among neurons crucially involved in memory maintenance during the middle stages of aging. This decline hints at a disruption in the recurrent circuits essential for working memory retention.

Optogenetic manipulation of the mPFC in both middle-aged and young mice provided compelling evidence that aging renders working memory susceptible to activity perturbations. This vulnerability underscores the fragility of cognitive processes in the face of aging-related alterations within the prefrontal circuitry.

Taken together, our findings shed light on how aging impacts prefrontal circuit function, particularly in working memory processes. By uncovering these critical changes, our study offers valuable insights into the early stages of cognitive aging and the mechanisms underlying age-related cognitive decline.

HFSP reference number: LT000187/2012-L
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2012
Fellow: KAMIGAKI, Tsukasa
Host supervisor: DAN, Yang

THE FORMATION, EXPANSION AND DISSOLUTION OF A-SYNUCLEIN INCLUSIONS IS MODULATED BY A GENETIC NETWORK ACTING THROUGH PHASE SEPARATION

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Abstract

Introduction: α-synuclein (αSyn) accumulates in the brains of patients with Parkinson's disease (PD) and forms inclusions in neurons called Lewy Bodies (LBs). LBs initially form in the brainstem and, as the disease progresses, are also observed in rostral regions. It has been hypothesized that this apparent spread of pathology is caused by the prion-like cell-to-cell transfer (propagation) of αSyn.

Results: To understand the mechanism underlying the propagation of αSyn, we did a high throughput screen. We cloned a construct encoding GFP-T2A-αSyn-RFP, with which we transiently transfected a HEK QBI cell line overexpressing wild type αSyn. The translated protein is cleaved at the 2A position, thus producing two independent proteins: GFP and αSyn-RFP. The latter then transfers to neighboring cells and the populations can be identified based on their colors: donor cells are RFP+GFP+ and recipient cells are RFP+GFP-. We used this model system to complete a genome wide, imaging based, arrayed siRNA high throughput screen that identified 38 genes whose knock down modifies the propagation of αSyn. Several of those genes have been implicated in the pathogenesis of neurodegeneration. Weighted gene coexpression network analysis (WGCNA) using gene expression data from multiple regions from healthy human brains showed that the 38 genes co-cluster with known PD genes in the same gene expression modules more frequently than expected by random chance. Follow up experiments in a novel tissue culture model system showed that αSyn molecules are mobile within inclusions as tested by FRAP experiments. This is consistent with the inclusions being liquid condensates formed via phase separation. Knock-down of two of the 38 genes increases the number and decreases the size of αSyn inclusions by modulating phase separation. One of those genes is also involved in pathways regulating the biogenesis of lipid droplets, and its knock-down affects the co-condensation between αSyn and lipid droplets. Experiments in iNeurons and molecular dynamic simulations are in progress to determine whether the underlying protein networks are enriched in intrinsically disordered proteins, and whether dysregulation of phase separation on a larger scale is the common mechanism underlying αSyn dysregulation in PD.

Conclusion: Our findings suggest that there is a link between αSyn aggregation, propagation and phase separation, and underlying pathways are regulated by genetic networks that dysfunction in PD.

HFSP reference number: LT001044/2017
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2017
Fellow: KARA, Eleanna
Host supervisor: AGUZZI, Adriano

ENERGY LANDSCAPES OF MUSCLE-TENDON DYNAMICS

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Abstract

Muscle is the primary animal motor, and the physical limits to muscle mechanical output consequentially have important implications for the limits to locomotor performance. It is well established that the mechanical performance of muscle can be altered by the presence of elastic tendons that are mechanically in-series. Here, we study the effect of this in-series elasticity from a fundamental physical perspective, and show that the resulting dynamics are governed by three dimensionless numbers: the physiological similarity index, which quantifies the ratio between the maximum kinetic energy and work capacity of muscle; the reduced stiffness, which quantifies the ratio between tendon elastic energy capacity and muscle work capacity; and the Galantis-Woledge number, which quantifies the ratio between the muscle kinetic energy and the tendon elastic energy capacity. These three numbers define an energy landscape that delineates regions where the presence of the tendons enhances or reduces muscle mechanical output. The size of the region where tendons enhance mechanical output decreases with animal size, suggesting that muscle-tendon units need to be more tightly tuned in large animals; eventually, at some critical size, tendons seize to offer a benefit over direct actuation, and muscle performance can no longer be enhanced.

HFSP reference number: RGY0073
HFSP Award category: ALUMNI Young Investigator Grant
HFSP Award year: 2020
Principal Investigator: LABONTE, David (UK)
Co-Investigators: BACCA, Mattia (Canada), HOLT, Natalie (USA).

ANTIVIRAL INNATE IMMUNE MEMORY IN ALVEOLAR MACROPHAGES FOLLOWING SARS-COV-2 INFECTION

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Abstract

Pathogen encounter results in long-lasting epigenetic imprinting that shapes diseases caused by heterologous pathogens. The breadth of this innate immune memory is of particular interest in the context of respiratory pathogens with increased pandemic potential and wide-ranging impact on global health. Here, we investigated epigenetic imprinting across cell lineages in a disease relevant murine model of SARS-CoV-2 recovery. Past SARS-CoV-2 infection resulted in increased chromatin accessibility of type I interferon (IFN-I) related transcription factors in airway-resident macrophages. Mechanistically, establishment of this innate immune memory required viral pattern recognition and canonical IFN-I signaling and augmented secondary antiviral responses. Past SARS-CoV-2 infection ameliorated disease caused by the heterologous respiratory pathogen influenza A virus. Insights into innate immune memory and how it affects subsequent infections with heterologous pathogens to influence disease pathology could facilitate the development of broadly effective therapeutic strategies.

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https://orcid.org/0000-0003-3478-5304

HFSP reference number: LT000203/2021-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: LERCHER, Alexander
Host supervisor: RICE, Charles M

REVEALING THE MOLECULAR MECHANISM OF PILI NANOFILAMENTS HIDDEN BACTERIAL HAIRS AS AN ON-OFF SWITCH TO POWER NATURE'S 'ELECTRIC GRID' BY CONTROLLING THE SECRETION OF ELECTRON CONDUCTIVE NANOWIRES: ASSEMBLY MACHINERY, FUNCTIONS, AND ELECTRON PATHWAYS

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Abstract

Electron transfer is central to all life processes. To avoid damage, organisms have evolved strategies to eliminate the surplus of electrons created by their metabolic processes. Most of these strategies involve electrons being transferred to soluble oxygen-like electron acceptors, which act as electron sinks. However, microbes that live in areas with limited or no oxygen, such as those that reside in the deep ocean, in soil, or in the human body, have evolved strategies to export electrons to extracellular acceptors such as minerals or other bacteria. *Geobacter* uses long thin conductive filaments called "nanowires" to export electrons. Nanowires are fundamental to global environmental processes including degradation of methane, a major greenhouse gas (1). *Geobacter* nanowires have intrigued the scientific community since they were first identified in 2002. Until recently nanowires were thought to be Type IV pili (T4P), polymers of the PilA-N pilin subunit, in part because T4P are required for electron transfer. However, Malvankar's work demonstrates that PilA-N pairs with a second protein, PilA-C, to form a T4P that is structurally inconsistent with electron transfer (2) and *Geobacter* produce additional filaments comprised of cytochrome subunits that could allow electron transfer through a chain of heme groups (3). For this HFSP project we will test our hypotheses that (i) these cytochrome filaments are the electron-conducting nanowires, and that (ii) the role of T4P in electron transfer is to secrete cytochrome subunits, which then assemble into nanowires on the bacterial surface. T4P would function as Type II secretion systems (T2SS), whereby pilin subunits form a periplasmic "endopilus" that acts as a piston to secrete molecules across the outer membrane. The project brings the PI together with experts in T4P (Craig), T2SS (Francetic) and cytochromes (Salgueiro) to explore the structure, assembly, and electron transfer mechanism of nanowires and to evaluate their role in bacterial respiration, communication, and pathogenesis. By combining experimental and computational studies, our team is addressing three key questions: How do microbes build & use nanowires? How are electrons transferred from the bacterial cytoplasm to surface-displayed nanowires? Can nanowire conductivity be tuned using light, and electric-fields to control bacteria?

Web: https://malvankarlab.yale.edu/

HFSP reference number: RGP017/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2024
Principal Investigator: MALVANKAR, Nikhil (USA)
Co-Investigators: CRAIG, Lisa (Canada), FRANCETIC, Olivera (France), SALGUEIRO, Carlos (Portugal)

MAINTENANCE, HOMEOSTASIS AND HEREDITY OF MITOCHONDRIA AND THEIR GENOMES

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Abstract

Mitochondria contain their own minimal genome, and undergo their own life cycle, asynchronous from the host cell cycle. The mito-cycle includes growth, mitochondrial DNA (mtDNA) replication, fission, and fusion. These processes require control to ensure distribution of mtDNA between mitochondria, and mitochondria between cells. Fission and fusion contribute to this regulation. In yeast and mammalian cell models, semi-regular spacing of mtDNA-containing nucleoids has been reported, and underlies controlled inheritance of genetic material between mitochondria. On the other hand, the harsh chemical environment inside mitochondria, rich in reactive oxygen species (ROS), makes mtDNA and matrix proteins susceptible to damage, and thus quality control to remove damage via mitophagy is another critical aspect of the mitochondrial life cycle. Yet, it is so far unknown how control – of nucleoid organization, copy number, or damaged material -- is achieved.

Our project has discovered that additional shape transformations contribute to the control principles of mitochondrial and mtDNA replication and segregation that ensure their inheritance at the organellar and cellular level. We describe how the pearling shape transition occurs in individual mitochondria, and quantify its impact. We also describe new tools we have developed to investigate this, and to discover new control mechanisms.

HFSP reference number: RGP0038/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: MANLEY, Suliana (Switzerland)
Co-Investigators: BADRINARAYANAN, Anjana (India), MARSHALL, Wallace (USA), PAULSSON, Johan (USA)

GETTING IN SHAPE—UNRAVELING THE MORPHODYNAMICS OF MICROBIAL COLLECTIVES

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Abstract

In nature, microbial organisms often self-organize into spatially structured communities, with distinct groups of cells occupying distinct spatial domains in 3D space. This spatial arrangement significantly influences diverse biological functions, including stability, nutrient access, and diversity; and yet, how exactly multicellular microbial communities get their shape and spatial structure remain poorly understood. Here, we first study how growing 3D bacterial colonies get their shape, a morphodynamical process that remains underexplored despite the prevalence of 3D environments in nature, e.g., soils and hosts. Using experiments in transparent 3D granular hydrogel matrices, we show that dense colonies generically become morphologically unstable and roughen as they consume nutrients and grow beyond a critical size—eventually adopting a characteristic broccoli-like morphology independent cell type and environmental conditions. This behavior reflects a key difference between 2D and 3D: while a 2D colony may access the nutrients needed for growth from the third dimension, a 3D colony inevitably becomes nutrient limited in its interior, driving a transition to unstable growth. We elucidate this instability using a continuum model that treats the colony as an "active fluid" whose dynamics are driven by nutrient-dependent cellular growth. We find that when all dimensions of the colony substantially exceed the nutrient penetration length, nutrient-limited growth drives a 3D morphological instability that recapitulates essential features of the experimental observations. Additionally, we extend our work to unveil the morphodynamics of multispecies communities, in which different microbes form monoclonal domains that compete for space and resources. What determines the shape of the interface between such domains—which in turn influences the interactions between cells and overall community function? Using a related model, we establish quantitative principles describing when different interfacial behaviors arise, and find good agreement both with the results of previous experimental reports as well as new experiments performed here. Altogether, our work thus provides a framework to predict and control the organization of proliferating colonies—as well as other forms of growing active matter, such as tumors and engineered living materials—in 2D and 3D environments.

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HFSP reference number: LT00035/2021-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2021
Fellow: MARTÍNEZ-CALVO, ALEJANDRO
Host supervisor: DATTA, SUJIT S.

DEATH & CHEMOTAXIS–UNRAVELING THE DYNAMICS OF BACTERIAL COMMUNITY MIGRATION IN THE PRESENCE OF PHAGES

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Abstract

Bacteriophages ("phages") are viruses that infect and kill bacteria; thus, phage-bacteria interactions shape microbiomes, with critical implications for agriculture, food, and medicine. However, laboratory studies typically use well-mixed cultures in test tubes or Petri dishes, which do not mimic nature's complexities. In nature, bacteria and phages exist in crowded 3D environments like soil and biological tissue, that impact behaviors. Furthermore, phage-bacteria interactions are typically studied at limited timepoints due to the experimental challenges of real-time analysis, further limiting understanding of how they influence microbiomes in practice. Here, we address this gap in knowledge using direct visualization of phage-bacteria interactions in transparent crowded matrices composed of packed hydrogel microparticles. We use confocal microscopy to visualize the real-time dynamics of motile *Escherichia coli* populations encountering lytic T4 phages as a function of the initial spatial distributions and concentrations. Additionally, we develop a theoretical framework that can predict our experimental observations, thereby helping to reveal the profound impact phages have on bacterial population dynamics.

HFSP reference number: LT00035/2021-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2021
Fellow: MARTÍNEZ-CALVO, ALEJANDRO
Host supervisor: DATTA, SUJIT S.

THREE SIDES OF THE SAME COIN: UNIFYING CONTEXT-DEPENDENCIES OF ECOLOGICAL INTERACTIONS

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Abstract

Ecological outcomes are shaped by feedbacks between organisms and their environment. For example, growth of a tree's canopy reduces light availability for plants in the understory, while some corals release toxins into their surroundings to kill nearby competitors. In assessing such feedbacks however, we are faced by the challenge that environmental factors are typically much more difficult to measure than the organisms that are changing them. Nowhere is this clearer than in microbial communities, in which growth-limiting environmental factors may include unknown secretions and breakdown products. Given the complexity of this environmental 'dark matter', it has been tempting to abstract out the environment in ecological models and treat the environmentally-mediated impact of species on each other as fixed. However, in recent decades it has become clear that these species-species impacts – or interactions – are strongly dependent on the environmental, spatial and temporal context in which they occur. Here, I will discuss new experimental and theoretical results that unify these context-dependencies, demonstrating how they arise inevitably from the very organism-environment feedbacks that mediate the interactions. Apparently disparate phenomena arising in different experimental settings – such as the spatial distribution of species in microfluidic systems and changes in growth rate in batch culture systems – are shown to arise out of the same underlying ecological processes. We also illustrate how knowledge of the mechanistic basis of interactions allows their context-dependent changes to be predicted. We plan to use this framework to deepen our understanding of environmental dark matter, exploiting a formal mapping between key ecological parameters to make use of high-throughput techniques that allow manipulation of dozens of environmental factors simultaneously.

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HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: MEACOCK, Oliver
Host supervisor: MITRI, Sara

SEPTIN BINDING TO SHIGELLA INDUCES A STRESS RESPONSE TO RESTRICT GROWTH

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Abstract

Shigella is a human pathogen and paradigm of cellular microbiology whose investigation has enabled landmark discoveries in infection and cell biology. Septins, a family of cytoskeletal proteins, form hetero-oligomeric complexes that assemble into polymers. Work has shown that septin cages form around intracellular bacteria and limit their dissemination. How septin filaments interact with bacterial surfaces for antibacterial activity, and if this depends on septin filament composition, are poorly understood.

In this work, we reconstitute septin-*Shigella* interactions in vitro using purified protein complexes to investigate (i) biophysical determinants of septin-bacteria interactions, (ii) how bacteria respond to septin binding, and (iii) outcomes of septin binding on bacterial physiology. We investigated two different septin complexes (hexamer and octamer) and found that they have different affinities for bacterial surfaces in a concentration dependent manner. To study the impact of septin-bacteria interactions, we measured the amount of volume growth in *Shigella* cells +/- septin binding. Strikingly, cell elongation rate is reduced by 20% in septin-bound bacteria, consistent for both filament types. We hypothesized that septin-mediated growth restriction is due to changes in bacterial physiology reflected in their transcriptional profile. In agreement, RNA sequencing revealed >30 significantly up- and down- regulated genes in bacteria recruiting septins. Pathway enrichment analysis showed septin binding induces a stress response by an unknown mechanism. We also found virulence factors to be upregulated, suggesting bacteria respond to septin binding by inducing pathogenic activity. We hypothesized that septin filaments on bacteria may act as physical barriers that restrict growth, causing modifications on the cell envelope and consequently triggering a stress response. Visualization of septin architecture on the bacterial surface with STORM confirms filaments are associated with the bacterial surface and reveals that septins form short filaments that are densely distributed along the long axis, supporting a role in inducing cell-envelope damage.

Our work reveals that septin binding, in the absence of other host factors, has antimicrobial properties that restrict bacterial growth. Considering that septin-bacteria interactions can act as a danger signal to stimulate cell-autonomous immunity, these findings transform our fundamental understanding of septin biology and host defence.

HFSP reference number: LT000436/2021-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: OZBAYKAL GULER, Gizem
Host supervisor: MOSTOWY, Serge

MULTIPLE SEROTONIN RELEASE MODES UNDER DISTINCT MOLECULAR CONTROL

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Abstract

Serotonin controls brain functions including mood, affection, and reward. Modulating serotonergic activity is used to treat anxiety, depression, and other brain disorders. Serotonin is thought to act as a volume transmitter, though the spatial and temporal scales of its transmission are not well understood. At classical synapses, position, timing and extent of neurotransmitter release are controlled by the active zone, a specialized protein complex. Here, we identified spatiotemporally distinct serotonin release modes in striatal serotonin axons that are mediated by specific protein machineries. To assess the functional dynamics of serotonin release, we imaged serotonin in striatal brain slices using an optical serotonin sensor, GRAB5-HT. Paired-pulse stimulation experiments suggested a high initial release probability of serotonin release and spatiotemporally distinct release components were detected. These properties predict that active zone-like protein machinery controls serotonin release properties. To identify the underlying molecular architecture, we utilized 3-D structured illumination super-resolution microscopy. We found that the active zone proteins Bassoon and Munc13 were present in ~30% of the striatal serotonin varicosities. To determine whether the proteins that mediate spatial precision and rapid release dynamics at synapses are needed for serotonin release, we probed the contribution of the active zone proteins Munc13 and RIM, and fast calcium sensor Synaptotagmin-1 using conditional mouse gene knockout. We found two forms of serotonin release with specific protein requirements. The first component was composed of serotonin hotspots, and RIM and Synaptotagmin-1 were essential for this form of release. A second component lacked a topographical organization and persisted without RIM or Synaptotagmin-1. Instead, it was partially sensitive to the deletion of Munc13, which also reduced the number of hotspots. In summary, our results indicate that there are multiple modes of serotonin release under specific molecular control. These spatiotemporally distinct release modes may play a key role in how serotonin modulates striatal circuits and behavior.

HFSP reference number: LT0004/2022
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: ÖZÇETE, Özge Demet
Host supervisor: KAESER, Pascal

ACTIVE MATTER MODEL INSPIRED BY T-CELL DYNAMICS IN LYMPH NODES

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Abstract

T-cell surveillance within lymph nodes (LNs) is essential for the adaptive immune response, facilitating the recognition of and reaction to pathogens. In exploring T-cell dynamics in high-density scenarios, our study leverages an abstracted active matter physics model to elucidate emergent dynamics and collective behaviors, while deliberately omitting detailed cell and biochemical mechanisms.

The 2D model simulates self-propelled particles—mirroring T-cell properties—with features like area-conserving shape changes (from circles to "pills" and back), shape-correlated active speed oscillations, and interaction through repulsion and adhesion in an overdamped regime.

A squared domain with periodic boundaries conditions encompasses a stationary particle representing an antigen-presenting cell (APC) at its center, surrounded by active particles,. Key to our analysis is the number of unique T-cell-to-APC encounters (*Ne*), serving as our system's primary performance metric.

Considering the system's particle quantity as the principal variable, our simulations uncover optimal densities at which Ne is maximized. Moreover, by comparing shape-changing particles with circular ones, we observe the added value of shape adaptability in enhancing particle motility at increasing density values.

Besides *Ne*, other metrics like Mean Squared Displacement, alignment probabilities (*Q(r)*), absolute values of mean velocities, mean speeds, and specific flow rates (borrowed from vehicular and pedestrian traffic science) were analyzed. The behavior of Ne, displaying a maximum value at optimum densities, cannot be captured by other observables except for the specific flow rate. It's worth mentioning that this observable, not usually considered in cell mobility studies, may prove useful, underscoring the value of interdisciplinary approaches.

This study not only establishes a framework for unveiling the emergent behavior of T-cells in high-density environments within lymph nodes (LNs) but also underscores the critical role of shape adaptability in enhancing cell motility. Furthermore, the design of the model allows for straightforward extension to 3D configurations, highlighting the broad applicability and potential of such abstracted approaches in analyzing many-body biological systems.

HFSP reference number: RGP0053/2020
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2020
Principal Investigator: TEXTOR, Johannes (The Netherlands)
Co-Investigators: MANDL, Judith (Canada), PARISI, Daniel (Argentina),

BIOINFORMATIC INVESTIGATIONS REVEAL A COMPLEX EVOLUTIONARY HISTORY OF ARTHROPOD INTERMEDIATE FILAMENTS

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Abstract

Intermediate filaments (IFs) are a large family of proteins that provide structural support for eukaryotic cells. They include the nuclear lamins, which form a supportive mesh within the eukaryotic nucleus, and cytoplasmic intermediate filaments (cIFs), which are crucial parts of the cellular "cytoskeleton" and constitute the bulk material for animal adaptations like nails, hair, and horns. cIFs are thought to have originated in the last common ancestor of bilaterally symmetrical animals (Bilateria) when a nuclear lamin gene duplicated and acquired a new localization in the cytoplasm. Despite their ancient origin and crucial functions, it has long been assumed that cIFs are absent from arthropods, the most numerous and diverse phylum of animals on the planet (including insects, crustaceans, and spiders). Such an idea, however, has been concluded based on the studies in a few model arthropod species. To comprehensively test the hypothesis of cIF loss from arthropods, we aim to combine cutting edge computational and cell biological techniques to search for cIFs in a broader group of arthropods. To date, we have leveraged novel computational search criteria combining sequence similarity, predicted protein structure, and predicted cellular localization to uncover putative cIF-like genes in 496 arthropod species including spiders, flies, springtails, and copepods. Phylogenetic analysis shows that most of these novel cIFs are most closely related to nuclear lamin sequences from the same clade, suggesting multiple instances of convergent evolution of cIFs from lamins in distinct arthropod groups. Intriguingly, here we also uncovered another group of the putative cIFs that are more evolutionarily related to other animal cIFs, and this result possibly suggests the retention of ancient bilaterian cIFs in certain arthropod lineages. With this list of cIF candidates, we selected three arthropod laboratory models, including the cricket *Gryllus bimaculatus*, the spider *Parasteatoda tepidariorum* and the fungus gnat *Bradysia coprophila*, for our future molecular and functional genetic examination. Overall, our results seem to suggest a complex evolutionary history of arthropod cIFs that might involve multiple independent cIF evolutionary events, as well as lineage-specific retention of the canonical cIF genes.

HFSP reference number: RGP0041/2022
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2022
Principal Investigator: TOMANCAK, Pavel (Germany)
Co-Investigators: HEJNOL, Andreas (Germany), HEISENBERG, Carl-Phillip (Austria), EXTAVOUR, Cassandra G (USA of America)

THE TRANSCRIPTIONAL PROGRAM OF GOLGI BIOGENESIS

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Abstract

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 combined high precision 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 asked whether genes encoding different components of the Golgi activated simultaneously or sequentially and how this activation correlates with structural and functional changes in the forming Golgi apparatus.

We found that cells building the new Golgi transactivate more than 100 Golgi genes that encode different classes of Golgi components including structural proteins, glycosylation enzymes and membrane tethering/trafficking complexes. Upregulation of these genes occurred in a coordinated manner and coincided in time with the recovery of the typical stack architecture and reactivation of transport through the newly-forming Golgi organelle. We also noted that activation of Golgi genes was not compartment-specific as induction of cis-, medial- and trans-Golgi genes occurred simultaneously. Thus, our findings suggest that orchestrated transactivation of various Golgi genes supports de novo biogenesis of the Golgi by providing building blocks for morphogenesis of the Golgi stacks and induction of their functional activities.

Finally, to assess the physiological relevance of our findings, we asked whether a similar transcriptional Golgi signature could be detected during differentiation of B lymphocytes to plasma cells, whose Golgi grow significantly in size to handle the massive flux of the newly synthetized antibodies. Indeed, analysis of RNA-seq data revealed that the Golgi transcriptome in differentiating B lymphocytes exhibited striking similarity to the cells forming the Golgi de novo. This suggests that the transcriptional program of Golgi biogenesis is used by cells in physiological contexts for adaptation to elevated cargo load in the secretory system.

HFSP reference number: RGP0046/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021

TRANSCRIPTIONAL PRIMING FACILITATES THE POLE CELL TO GERM CELL TRANSITION DURING DROSOPHILA EMBRYOGENESIS

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Abstract

Cell state transitions play central roles in development, homeostasis, and disease. On one hand, transitions from one cell state to another are essential for cells to differentiate (e.g. stem cell transition into a daughter cell), to respond to stress (e.g. lymphoid progenitor into a T cell or B cell), or to undertake distinct behaviors (e.g. epithelial-to-mesenchymal transitions). Conversely, dysregulation of mechanisms regulating cell state transitions can result in disease and cancer (e.g. autoimmune syndromes, metastasis). Here, we uncovered and characterized a novel cell state transition during Drosophila melanogaster embryonic germ cell development that enables pole cells to transition to primordial germ cells. Using single-cell transcriptomics of isolated germ cells at three developmental stages (blastoderm, germband elongation, germband retraction) we identified two germ cell trajectories: 1) A set of germ cells that comprise of pole cells, migrating germ cells, and coalescing germ cells. 2) A set of germ cells isolated from the migrating and coalescing timepoint that are characterized by retaining a maternal transcriptional profile, where rapidly degraded mRNAs such as nanos, pgc, and germ cell-less are maintained at high-levels. Next, we characterized the progression of these germ cells in vivo and found differential expression of maternal enriched mRNAs (e.g. nanos) is first apparent after germband elongation and by germband retraction germ cells that fail to degrade their maternal mRNAs have condensed nuclei, indicating they are undergoing cell death. These germ cells, deemed the “lost children” represent the earliest quality checkpoint during the germ cell life cycle. Next, we speculated that the retention of a maternal program in the “lost children” suggest a failure in cell state transition that entrenches these germ cells into a “naïve” pole cell state. A major driver of cell state transitions is transcriptional priming. To test whether “lost children” fail to undergo a maternal-to-zygotic transition we stained embryos at the blastoderm and germband elongation stage with a phosphorylated RNA polymerase II antibody. At the blastoderm stage we found significant heterogeneity in germ cell transcriptional state, while at the germband elongation stage we found a negative correlation between germ cells expressing zygotic transcripts and germ cells retaining maternal transcripts. Overall, our data reveals unexpected heterogeneity in germ cell states during early embryogenesis, where previously pole cells were believed to have uniform totipotent developmental capacity, while now a subset of germ cells exist that cannot undergo a cell state transition, remaining forever a pole cell.

HFSP reference number: LT0053/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: RAJAKUMAR, Arjuna
Host supervisor: Lehmann, Ruth

SELECTION OF OPTIMAL HYDRAULIC MODES IN THE NETWORK FORMING SLIME MOLD PHYSARUM POLYCEPHALUM

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Abstract

The syncytial slime mold *Physarum polycephalum* builds a living hydraulic network to traffic organelles and resources across the entire organism. Cytoplasm is pumped through the network by rhythmic contraction and dilation of actomyosin skeleton within the walls of the network. Despite the lack of a central organizing center, the organism is capable of creating and maintaining global patterns of cytoplasmic flow, and of adapting both the morphology of the network and the flows it carries, in response to encountering new food sources or to initiate migration. Here we report on our collaborative work to understand: 1. How large is the space of possible cytoplasmic flows? 2. How much of this space do real slime molds occupy. 3. How do networks select which cytoplasmic flows to create? Particularly, we report on our construction of a general mathematical framework for mapping the entire repertoire of flow modes a network can create, ranked by their efficiency in creating long range transport. Our method reveals both the richness of its repertoire of possible behaviors a network can use, but also that with surprising robustness, the organism selects a small number of modes that are optimally efficient at creating long range transport. We also report on our ongoing work to understand the mechanisms by which modes of cytoplasmic flow are selected, including the development of new tools for labeling and tracking signal-carrying nuclei that cross back-and-forth across the network.

HFSP reference number: RGP0001/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: ALIM, Karen (Germany)
Co-Investigators: ROPER, Marcus (USA), ROZEN, Daniel (Netherlands)

AN ANGIOGENIC SWITCH REGULATES THE TRANSITION FROM A WOUND HEALING TO A BLASTEMA STATE DURING AXOLOTL LIMB REGENERATION

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Abstract

The ability to therapeutically treat limb loss is a key goal of regenerative medicine. While mammals cannot naturally regenerate limbs, salamanders, including axolotls, are inherently capable of regrowing entire limbs. Limb regeneration requires the formation of a specialized wound epidermis and the subsequent formation of a blastema from activated stump progenitor cells. Blastemas contain multipotent cells which give rise to many of the tissues of the regenerated limb. How regeneration differs from wound healing which lacks blastema formation, such as mammalian wound healing, remains mysterious. The role of nerves and other cell types within the blastema is becoming well established, but how vasculature contributes remains unclear. Vasculature largely forms via angiogenesis, which is primarily mediated through vascular endothelial growth factor (VEGF) signaling. Here, we show that when VEGF signaling is pharmacologically blocked, amputated axolotl limbs fail to transition out of a wound-healing response, and blastema cells fail to coalesce to form a functional blastema despite being specified. Under these conditions, genes associated with wound healing and scar formation are upregulated. Together, these data indicate that angiogenesis may trigger the switch from a wound-healing program to a blastema-formation response, an essential feature of successful limb regeneration.

HFSP reference number: LT0014/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: SAVAGE, Aaron
Host supervisor: WHITED, Jessica

THE APHRODISIAC GUT: DISSECTING THE MULTI-LEVEL CONNECTION INVOLVED IN YEAST MATING PROMOTION IN WASP GUTS THROUGH A MULTI-DISCIPLINARY COLLABORATION

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Abstract

The natural evolution and ecology of a familiar microorganism, *Saccharomyces cerevisiae*, known as the "baker's yeast", is far from being fully understood. Recent observations have shown that intra-strain sexual reproduction (outbreeding) of this yeast, rarely observed in natural settings, is promoted within the intestine of a natural *S. cerevisiae* vector: social wasps. The process toward outbreeding requires multiple conditions to be met: diploid cells should face stresses promoting sporulation to form haploid cells which, upon restoration of suitable environmental settings, become sexually active and, if released from the ascus (a container of spores of the same strain) and exposed to sexually active cells of another strain for a sufficiently long time, can finally outbreed. Our multi-disciplinary group is gathering fundamental insights to disclose the feature characterizing the wasp gut environment and promoting the outbreeding, potentially through a sequence of peculiar drastically different chemical and mechanical stresses. Microfluidic analyses showed that the physical forces imposed by wasps' peristaltic intestine motility are not sufficient to break the ascus and hence release the spores for outbreeding. This observation led to the exploration of lytic activities eventually present within the wasp gut. The analysis performed by exposing yeast ascus to the wasp intestine content revealed not only that the gut lumen induced the lysis of the ascus, but also that this effect had different intensities in different intestine compartments, hence highlighting the need to include in the puzzling portrait both the chemical and mechanical stresses and the structural conformation of the wasp gut to model the factors necessary for outbreeding promotion. COMETS, a platform for performing computer simulations of metabolism in spatially structured microbial communities, was implemented for this intent by including the structural conformation of insect intestines and all the metabolic pathways associated with yeast sporulation. The information gathered by means of physiological, physical, and chemical characterization of the wasp intestinal environment, achieved also thanks to ad hoc developed sensors for the detection of chemical features known to be involved in yeast sporulation and mating (e.g. nitrogen and nutrient starvation), will be introduced into COMETS to permit reconstructing the intriguing puzzle of yeast outbreeding promotion within the wasp gut.

HFSP reference number: RGP0060/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: Stefanini, Irene (Italy)
Co-Investigators: Segrè, Daniel (USA), NEW, Elizabeth (Australia), POLIN, Marco (Spain)

ROBOTIC IMAGING REVEALS SUPPLY-CHAIN DESIGN OF PLANT-MYCORRHIZAL TRADE

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Abstract

Mycorrhizal fungi construct complex mycelial networks to collect and trade nutrient resources with plant roots. Like human-built commercial networks, these fungi face conflicting trade-offs in building networks that balance low construction costs with high geographic coverage and long-distance transport. Yet, how they navigate supply-chain design challenges is unknown. To monitor the construction of a living trade network, we built a custom-designed robot for high-throughput time-lapse imaging that could track>500,000 fungal nodes simultaneously. We then measured ~100,000 cytoplasmic flow trajectories inside networks, toward and away from roots. We found mycorrhizal fungi build networks as 'self-regulating' traveling waves: pulses of growing tips pull an expanding waveof nutrient-absorbing mycelium whose density is self-regulated by fusion. I will discuss the significance of this traveling-wave strategy within the context of symbiotic trade, and present the first evidence that these fungi, despite their diffuse and distributed anatomy, achieve network-level control of cytoplasmic flows to meet trade demands.

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HFSP reference number: RGP0029/2019
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2019
Principal Investigator: KIERS, Toby (Netherlands)
Co-Investigators: STONE, Howard A. (USA), SHIMIZU, Thomas (Netherlands), TOJU, Hirokazu (Japan)

SELFCURE: EVOLUTIONARY AND COGNITIVE PROCESSES UNDERLYING SELF-MEDICATION OF IMMUNE-CHALLANGED BATS

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Abstract

The medicinal properties of many plant species are used by a diverse range of animal taxa. Chimpanzees use hairy leaves when suffering from intestinal parasites, Kodiak bears use roots against ectoparasites, and monarch butterflies lay their eggs on milkweed that is toxic to protozoans infecting their offspring. These behaviors are suggestive of self-medication, as they provide an effective mechanism to minimize parasite load. But despite its eco-evolutionary importance, we lack clear understanding how behaviors associated with self-medication can evolve. Importantly, animals may either possess an innate mechanism to search for medicinal food items when health is challenged, or they may learn individually or through others to associate different foods with different cures. Neotropical fruit bats are a particularly interesting group to study the mechanism underlying self-medication. Like all bats, they harbor many parasites, feed on a wide variety of plants, and—due to their cognitive capacities and longevity—have high potential to learn to associate plants with their curative properties. Using state-of-the-art tracking techniques, AI-based individual recognition, and targeted and -omics approaches for characterizing immunity and parasites, we are investigating the mechanism(s) underlying self-medication in Neotropical fruit bats from the family Phyllostomidae. In our presentation, we will discuss the findings from our initial field expedition to the Panamanian rainforest, unveiling a novel wingprint technology we engineered. This technology facilitates the re-identification of bats based on distinctive patterns on their wing membranes akin to human fingerprints. Additionally, we will talk about the great opportunity funded by the Scientist4Scientist initiative, which facilitated the integration of Ukrainian scientists from the Bat Rehabilitation Center in Kharkiv into our project.

HFSP reference number: RGP002/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2023
Principal Investigator: BECKER, Daniel (US)
Co-Investigators: PAGE, Rachel (Panama), SIMON, Ralph (Germany)

HOW DO SOCIAL ANIMALS USE VOCAL COMMUNICATION TO COORDINATE GROUP BEHAVIORS?

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Abstract

Sociality is widespread in nature, but group living requires coordination. From achieving consensus about where to move, to banding together against common enemies, coordinated action requires communication to solve a range of tasks. In many cases, animals rely on sound to communicate, and many species have evolved sophisticated vocal communication systems that facilitate coordination. Despite the widespread importance of these collective behaviors, understanding how group coordination emerges remains a fundamental challenge that conventional approaches of behavioral field biology are not well equipped to address due to the difficulty of monitoring many individuals simultaneously. In the "Communication and Coordination Across Scales" project, we are combining longitudinal field observations with new bio-telemetry and machine learning tools to tackle this challenge. Using multi-sensor collars to track the movements, vocalizations, and behaviors of entire social groups simultaneously as they communicate and coordinate in their natural environment, we are gaining new insights into how individuals make decisions, how information is transmitted through groups, and ultimately how these decisions give rise to coordinated group behaviors.

We present full-group tracking data from three species of social mammals spanning a range of dispersion patterns: highly cohesive meerkats (*Suricata suricatta*), moderately-cohesive white-nosed coatis (*Nasua narica*), and widely dispersed spotted hyenas (*Crocuta crocuta*). Using these data, and through development of machine learning approaches allowing us to detect and classify animal vocalizations and behaviors, we show first results addressing several questions at the interface of communication and collective behavior. (1) Who has influence over group movement decisions and what is the role of communication in governing these decisions? (2) How does acoustic information flow through groups to mediate collective responses to threats? (3) When and why do groups split up and come back together, and what role does vocal communication play in mediating these fission-fusion dynamics? Ultimately, our goal is to search for common principles that may underlie communication and coordination dynamics across species, while also understanding how social and ecological constraints drive variation in the ways animals communicate and coordinate.

Web: https://www.movecall.group/

HFSP reference number: RGP0051/2019
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2019
Principal Investigator: STRANDBURG-PESHKIN, Ariana (Germany)
Co-Investigators: MANSER, Marta B. (Switzerland), HIRSCH, Ben T. (Australia), HOLEKAMP, Kay E. (USA), ROCH, Marie A. (USA)

SIGNAL-DEPENDENT REGULATION OF NF-KB ACTIVITY

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Abstract

The NF-κB family of transcription factors functions as the central hub integrating and interpreting a wide range of signals. NF-κB is normally inactive in cells and is bound to specific inhibitor proteins (IkBs) in the cytoplasm. Various stimuli, such as infection, inflammation, or stress activate NF-κB, which is released from the inhibitor proteins and translocates to the nucleus, where it binds to DNA and regulates the expression of genes involved in various cellular processes. NF-kB is using a combination of strategies, such as sophisticated signaling pathways often cross-talking with other cellular pathways, negative feedback loops and cell-specific transcription mechanisms, to orchestrate appropriate cellular responses. We identified a novel NF-kB signaling component called CYLD (cylindromatosis tumor suppressor), which binds and regulates the NF-kB activating IKK kinase complex. CYLD is a de-ubiquitinase attenuating IKK activity. CYLD mutations lead to sustained NF-kB activation and tumorigenesis implying that it functions in a negative autoregulatory feedback loop on NF-kB activation. As NF-κB signaling pathways interact with other pathways allowing more refined responses depending on the combination of signals a cell receives, we investigated how NF-κB suppresses TNF-induced cell death. We found that TNF induced c-FLIP to prevent apoptosis and also stimulated persistent MAPK activation in cells lacking NF-κB. This prolonged MAPK activation is due to the expression of IL-11. We found that the limiting amounts of NF-kB result in the stochastic expression of most immune genes. Cells cope with limiting amounts of NF-κB by capturing the factor in three specialized genomic elements termed NRCs (NF-κB Reception Centers) and delivering it directly to its target genes via stochastic interchromosomal interactions to initiate transcription. These interactions are mediated by the co-bound transcription factor ThPOK, which upon oligomerization mediates the assembly of hubs bearing coordinately expressed and NF-kB-regulated genes. Lack of ThPOK leads to NRC hub disassembly and to sporadic and random gene expression. Through extensive structural analyses, we have significantly enhanced our understanding of the mechanisms dictating the selective binding of various NF-kB dimers, thus elucidating why even subtle differences in DNA sequences can favor one NF-kB dimer over others. We also examined how the IkB inhibitors interact with NF-kB to modulate its activity and how the IKK kinase complex is activated by diverse stimuli. Taken together, our studies have shed new light on the molecular mechanisms by which NF-kB is aberrantly activated to induce pathogenic programs and exploring strategies to reverse this aberrant activity.

HFSP reference number: RGY0276/2001-M
HFSP Award category: ALUMNI Research Grant
HFSP Award year: 2001
Principal Investigator: THANOS, Dimitris (USA-Greece)
Co-Investigators: MOSIALOS, George (Greece), NAKANO, Hiroyasu (Japan), GHOSH, Gourisankar (USA)

AVIAN SENSING OF AIRFLOW THROUGH FEATHER VIBRATION

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Abstract

Many birds are extremely agile flyers and integrate many different sensory modalities to control their flight. Sensing the pattern of air over their wings is thought to be an important sensory input. It is known that wing feathers vibrate in relation to the airflow over them and that 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 peripheral nervous system and then processed in the brain. This multidisciplinary project looks at these three stages of information processing.

Using zebra finches (*Taeniopygia guttata*) as our study species we have characterised the first stage, 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 frequencies and these filtering properties change throughout wing morphing.

Moving to the second stage, the peripheral nervous system, we are in the process of characterising the neuroanatomy using histology to measure nerve distribution properties and electrophysiology to characterize the response properties of the mechanoreceptors.

Finally, in the third stage we are characterising the flight behaviour and wing kinematics of zebra finch to correlate flight behaviour and activity in the birds central nervous system. We have already identified areas of interest in the brain using a combination of electrophysiology and activity-dependent protein expression studies.

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.

HFSP reference number: RGP0068/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: WINDSOR, Shane (UK)
Co-Investigators: PERKEL, David (USA), WOOLLEY, Sarah (Canada)

NUCLEAR ENVELOPE BUDDING AS AN ALTERNATIVE ROUTE TO EXPORT MRNA

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Abstract

The nuclear pore complex (NPC) has long been considered the sole gateway for macromolecule transport across the nuclear envelope (NE). However, emerging evidence suggest that NE budding (NEB) could function as a non-canonical route, facilitating the export of oversized cargoes, such as viral nucleocapsids and large ribonucleoproteins.

My work investigates NEB as an alternative pathway for endogenous cargo export in mammalian cells, with a specific focus on RNA transport.

I focused on myogenesis as an ideal condition for NEB to occur, where the expression and export of uniquely long transcripts are critical for sarcomere formation, and I applied a comprehensive approach combining electron and fluorescence microscopy. My work demonstrates that NEB occurs exclusively upon differentiation of C2C12 mouse myoblasts into myotubes. Single-molecule fluorescence in situ hybridization experiments highlight the preferential localization of large sarcomeric transcripts within NE buds. Moreover, using proximity proteomics to identify NEB protein cargo, I identified several nucleoporins and RNA export factors enriched within NE buds. I further investigated the relationship between NEB and NPC-mediated export. Analyses of various mRNA export factors revealed unexpected changes of expression levels and/or localization occurring during myogenesis, providing compelling evidence for an alternative export pathway.

Together, my results propose NEB as a novel mRNA export route, activated when exceptionally long sarcomeric transcripts are unable to be efficiently exported across the NE via NPC. This discovery represents an exceptional contribution in our understanding of RNA transport and muscle biology, and lays the groundwork to further investigate the molecular mechanisms regulating NEB.

HFSP reference number: LT000527/2021-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: ZAGANELLI, Sofia
Host supervisor: VOELTZ, Gia

POSTER TALKS

WHOLE-BRAIN NETWORK ANALYSIS FOR NEURONAL REPRESENTATION OF IMPRINTED COURTSHIP CUES

Stefano Zucca[1]; Alessandra Stella[1]; Sarah Zala[2]; Paolo Marcello Peretto[1]; Dustin Penn[2]; Serena Bovetti*[1]
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Abstract

Acquiring sensory-related memories during infancy is a process of social learning found in many species and it has a variety of different adaptive functions. For example, sexual imprinting is a process of instinctive learning during early postnatal development in which individuals acquire memories of the odor, vocalizations, and other characteristics of their parents (or siblings), and then utilize this information to select their mates as adults (Bateson, 1978, Yamazaki, 1976). Sexual imprinting has evolved in house mice and many other species, and yet surprisingly little is known about the underlying neuronal mechanisms. In rodents, male mice attract females using a combination of olfactory and acoustic cues, and past works in inbred mice showed that females have an innate preference for odors and vocalizations of a different strain, but only when reared with their father. Here by using whole-brain immunolabeling for the immediate early gene cFos, iDISCO tissue clearing and light-sheet fluorescence microscopy (Renier et al., 2016), we investigated how the mouse brain represents imprinted and novel sensory signals, and whether the resulting functional networks display hub features. Moreover, for generalization and ecological validation we compared results from laboratory mice (C57BL6J) to wild house mice (*Mus musculus musculus)*. We evaluated the number of cFos-positive neurons across all brain areas and compared brain activation after exposure to either imprinted or novel cues, in females reared with or without their father. To better characterize the recruitment of different brain networks, we calculated correlations across various brain areas and used mean-centered partial least squares correlation analysis to identify a set of brain regions functionally connected within the same experimental group. We combined such results by constructing weighted undirected graphs where we identified functional subnetworks and areas which acted as hubs in response to sensory cues. Overall our data highlight a differential recruitment of brain networks after the exposure to imprinted or novel male sensory cues, with novel cues recruiting more interconnected networks compared to imprinted ones. Moreover, we identified a subset of thalamic and hypothalamic areas able to discriminate between the two conditions. Current experiments are focusing on these key regions and aim to use functional approaches to precisely characterize neuronal responses in female mice during mate selection.

HFSP reference number: RGP0003/2020
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2020
Principal Investigator: BOVETTI, Serena (Italy)
Co-Investigators: PENN, Dustin (Austria), GIGAN, Sylvain (France).

CELL-STATE SPECIFIC REMODELING OF THE MALATE ASPARTATE SHUTTLE

Julia Stefanie Brunner*[1]; Anna E. Bridgeman[1]; Benjamin T. Jackson[1]; Katrina I. Paras[1]; Abigail Xie[1]; Paige K. Arnold[1]; Lydia W. S. Finley[1]

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Abstract

Mammalian cells require energy, biosynthetic precursors and reducing equivalents in order to survive and proliferate. To support essential redox metabolism, cells rely on electron shuttles to maintain continuous regeneration of electron carriers, but how electron shuttles are configured to meet the demands of different metabolic states is unknown. In particular, the malate aspartate shuttle (MAS) is a major electron shuttle that transfers reducing equivalents into the mitochondria and regenerates cytosolic NAD+ from NADH. In the cytosolic half of the MAS, glutamic-oxaloacetic transaminase (GOT1) consumes aspartate to form oxaloacetate, which is then reduced by malate dehydrogenase 1 (MDH1) to regenerate NAD+. Continuous engagement of the cytosolic half of the MAS therefore consumes aspartate at the expense of aspartate serving as an anabolic precursor for protein and nucleotide synthesis. Here, we present evidence that proliferating progenitor cells show low reliance on the cytosolic half of the MAS, most likely due to high demand for aspartate as a biosynthetic precursor. In contrast, cells undergoing differentiation assemble the full MAS, allowing them to meet redox demands imposed by a shift towards an increased oxidative state. Our study suggests that as cells differentiate, electron shuttles reconfigure to support the metabolic demands of differentiation.

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HFSP reference number: LT000200/2021-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: BRUNNER, Julia Stefanie
Host supervisor: FINLEY, Lydia

HIGHLY PARALLEL AND PROGRAMMABLE SINGLE-MOLECULE FORCE SPECTROSCOPY BY LIGHT-GUIDED PATTERNING

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Abstract

While advances in nucleic-acid sequencing have revolutionized biomedical research, corresponding developments in proteomic approach remains a challenge due to the lack of technology that can decipher proteomes in a highly parallel manner1. Unlike DNA that can be easily amplified with polymerase chain reaction (PCR) and detected with relatively low sensitivity instrumentation, proteins cannot be replicated and are considerably more complex, making protein analysis more challenging [1]. Single-molecule approaches, including multiplexed single-molecule force spectroscopy (SMFS) have emerged as an promising tool for proteome analysis. Our lab has recently demonstrated the precise distance fingerprinting of single molecules using DNA nanoswitch calipers actuated by mechanical force, which can enable the identification of proteins and the characterization of their post-translational modifications [2]. Various technologies such as magnetic tweezer, centrifuge force microscopy (CFM), and hydrodynamic force spectroscopy can be used to stretch biomolecules with beads and solid surface to apply forces to many proteins in parallel, with their molecular characteristics inferred from the positions of the beads [3]. Accordingly, the programmability and throughput of these technologies are inherently linked to the distribution and density of biomolecules or beads on the surface, with randomly or sparsely distributed beads leading to limited throughput. Here, we propose combining SMFS with precisely organized biomolecule patterning to enable spatially resolved highly parallel single-molecule force spectroscopy. Oligonucleotides (oligos) are patterned on the flow cell by employing 3-Cyanovinylcarbazole (CNVK) that can be covalently crosslinked upon illumination of UV (365 nm), with UV illumination patterned with a digital micromirror device (DMD). To demonstrate high programmability and throughput, we have realized diverse patterns of biomolecules such as square, and hexagonal lattices at a variety of densities. Furthermore, we have applied our patterning approach to single-molecule magnetic tweezers measurements to demonstrate the technology's capability of precisely organizing biomolecules for high-throughput force spectroscopy.

HFSP reference number: LT0005/2023-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2023
Fellow: CHOI, Hansol
Host supervisor: WONG, Wesley

A CONSERVED FERTILIZATION COMPLEX BRIDGES SPERM AND EGG IN VERTEBRATES

Victoria Deneke*[1]; Andreas Blaha[1]; Yonggang Lu[2]; Jonne Draper[1]; Clara Phan[1]; Karin Panser[1]; Alexander Schleiffer[1]; Laurine Jacob[1]; Theresa Humer[1]; Karel Stejskal[1]; Gabriela Krssakova[1]; Dominik Handler[1]; Maki Kamoshita[2]; Tyler Vance[3]; Xinyin Wang[3]; Elisabeth Roitinger[1]; Jeffrey Lee[3]; Masahito Ikawa[2]; Andrea Pauli[1]

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Abstract

FERTILIZATION, THE BASIS FOR SEXUAL REPRODUCTION, CULMINATES IN THE BINDING AND FUSION OF SPERM AND EGG. WHILE SEVERAL PROTEINS HAVE BEEN DEMONSTRATED TO BE ESSENTIAL FOR THIS PROCESS IN VERTEBRATES, THE UNDERLYING MOLECULAR MECHANISMS ARE POORLY UNDERSTOOD. HERE, WE PERFORMED AN ALPHAFOLD-MULTIMER SCREEN, WHICH IDENTIFIED A CONSERVED TRIMERIC SPERM COMPLEX COMPOSED OF THE KNOWN ESSENTIAL FERTILIZATION FACTORS IZUMO1 AND SPACA6, AND THE UNCHARACTERIZED PROTEIN TMEM81. WE DEMONSTRATE THAT TMEM81 IS ESSENTIAL FOR MALE FERTILITY IN ZEBRAFISH AND MICE. CONSISTENT WITH TRIMER FORMATION IN VIVO, IZUMO1, SPACA6, AND TMEM81 INTERACT IN ZEBRAFISH SPERM. STRIKINGLY, WE FIND THAT IZUMO1 AND SPACA6 FORM A COMPOSITE BINDING SITE FOR THE EGG FERTILIZATION FACTOR BOUNCER. OUR WORK PRESENTS AN INTRIGUING MODEL FOR FERTILIZATION ACROSS VERTEBRATES, WHERE A CONSERVED SPERM COMPLEX BINDS TO DIVERGENT EGG PROTEINS, BOUNCER IN FISH AND JUNO IN MAMMALS, TO MEDIATE SPERM-EGG INTERACTION.

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HFSP reference number: LT000455/2020-L
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2020
Fellow: DENEKE, Victoria
Host supervisor: PAULI, Andrea

DERIVING JERBOA INDUCED PLURIPOTENT STEM CELLS TO UNRAVEL THE MECHANISMS OF LIMB-SIZE CONTROL VIA INTER-SPECIES CHIMERAS

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Abstract

Evolutionary developmental biology (evo-devo) aims to understand how developmental mechanisms change during evolution. I am interested in the mechanisms and evolution of organ-size control. I use the vertebrate limb as a model because the spectrum of limb size and proportions provides a strong basis to study evolutionary diversity. Also, limbs are dispensable for embryo survival, enabling perturbation studies. I aim to define the mechanisms controlling limb growth using limb-specific inter-species chimeras (ISCs), by injecting stem cells from donor species of different limb size into early host mouse embryos. Recent studies showed that when regenerating limb blastemas are grafted from small to large axolotls, the resulting limbs grew to be bigger than in the donor(1). Moreover, manipulation of retinoic acid signalling from the flank was shown to alter the tempo and final size of the limb in avian species(2). I hypothesize that if early limb cells are placed within the signal environment of a different species, they will adapt their growth program accordingly, leading to a change in limb size, as compared to the donor. These chimeras will be analysed by using different methods such as 3D limb morphometry, immunostaining, transcriptomics, and chromatin-accessibility analyses to identify the gene regulatory networks involved in the control of limb size that are differentially active in donor limbs grown in normal donor bodies vs. host bodies. In our innovative approach we decided to use jerboa as our donor species, as they are roughly the same size as a mouse, but their feet are disproportionally long. However, since jerboas cannot be time mated, it is extremely difficult to get their embryonic stem cells. We, therefore, generated jerboa induced pluripotent stem cells (iPSCs) from embryonic fibroblasts (kindly provided by Prof. Cooper) using a viral vector that reprograms their genetic make-up and brings them to pluripotent state(3). This work was carried out under the guidance of Dr. Tsutsumi. I will discuss how the cells lines were established and confirmed as iPSCs by various experiments such as qPCRs, RNA-sequencing, immunostaining, and differentiation assays. These jerboa iPSCs will be used to complement blastocysts of *limbless* mouse models, so that the limbs of the resulting chimeras are exclusively derived from the jiPSCs. This research will generate more tools for the generation of iPSC and ISC, as well as provide new avenues for evo-devo research.

Web: https://www.rosellodiezlab.com/

HFSP reference number: LT0020/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: GOEL, Isha
Supervising host: ROSELLO-DIEZ, Alberto

ROBUST FOLDING OF THE EXPANDING PUPAL WING UNDER CONFINEMENT

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Abstract

During development, complex multicellular organisms construct organs by coordinating processes through space and time. In many cases, organisms or their constitutive parts temporarily develop under confined spaces, such as *Drosophila melanogaster* in the pupa. Little is understood about how these boundary conditions contribute to the shapes that arise in morphogenesis, much less on the cellular and tissue scale dynamics. Within the first day of *Drosophila* metamorphosis, the wing through a series of cellular deformations and divisions transforms from a single layer disc of cells to a two cell-layer sheet confined within a cuticle sac of similar cross-sectional area. The material composition at the interface between the two cell layers also changes during this time to allow the layers to remain apposed to one another [Diaz-de-la-Loza *et al*. (2018)]. Over the next few days, the wing expands in surface area fivefold through local buckling and folding while the cuticle sac remains static, with the initiation of folds occurring at the wing tip where it is no longer tethered to the bounding sac [Tsuboi *et al*. (2023)]. Still, remarkably little remains known about the nature of the folding, both at the cellular and tissue levels. For example, much is unknown about the duration of the fold process and whether the folding pattern is deterministic across individuals in certain regions and more variable in others. As the wing must be planar in adulthood, how the cells of this bilayer coordinate their expansion during folding without compromising the final wing structure is also unclear. Here, we report upon recent findings with the wing folding process, including the full propagation of the folding process across the entire wing and the location of deterministic and stochastic fold patterns across replicates. We also show that during this time, the constitutive cells are expanding their surface area and becoming progressively thinner. Finally, we show the recruitment of key mechanosensitive proteins at the interface of the two cell layers, suggesting an increase in mechanical tension to keep the layers apposed during folding. These macroscopic results provide the tissue-scale factors of folds and will help guide our future examinations of the subcellular factors that carefully guide cell deformation and tissue folding without compromising the integrity of a vital part of the winged insect body plan.

HFSP reference number: LT0016/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: HIROKAWA, Soichi
Host supervisor: LECUIT, Thomas

ROCK-PAPER-SCISSORS DYNAMICS BETWEEN MOBILE GENETIC ELEMENTS

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Abstract

One of the biggest questions in microbial ecology is how natural communities maintain species diversity. Theory predicts that nontransitive "cyclic" interactions between species can stabilize diversity. In a three-species community (A, B, C) such interactions resemble the game rock-paper-scissors (RPS): A beats B, B beats C, yet C beats A. Despite a large theoretical literature, experimental realizations of nontransitive interactions remain rare.

Here, we hypothesize that nontransitive interactions naturally arise in bacterial communities infected with multiple competing plasmids. Mathematical modeling suggests that a costly conjugative and a less costly immobile plasmid can coexist with a plasmid free strain, displaying stable oscillations in population size of the three strains. In this poster, we will detail predictions of the stability and dynamical behavior of this three-strain system, as well as our ongoing efforts to test these predictions *in vitro*. We are developing an experimental system with three *Escherichia coli* strains that differ only in their carriage of two competing antibiotic resistance plasmids. Successive 2- and 3-way competition experiments will allow us to study the stable states of this system over a range of environmental parameters.

Ultimately, this work will allow us to test existing theoretical predictions about the nature and importance of nontransitive interactions for community stability. Such insights can help engineer more stable synthetic communities with applications ranging from bioremediation to medical therapies. In addition, understanding plasmid diversity and maintenance is directly relevant to the study of bacterial colonization and infection of human and animal hosts, as well as the spread of antibiotic resistance.

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HFSP reference number: LT0045/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: HUISMAN, Jana Sanne
Host supervisor: GORE, Jeff

HFSP-240018

USING ELECTRON CRYOTOMOGRAPHY TO UNDERSTAND ACTINOBACTERIAL MEMBRANES

Rory Hennell James*[1]; Catalin Bunduc[2]; Sita Coenraads[2]; Gregor Weiss[3]; Wilbert Bitter[2]; Thomas Marlovits[1]
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Abstract

Mycobacteria are responsible for several globally relevant diseases, including tuberculosis, the world's most deadly infectious disease. Many strains of mycobacteria encountered in the clinic show extensive resistance to currently-available antibiotics, and therapies often require months-long treatment programmes with severe side-effects. Although they are found within the usually monoderm Gram-positive *Actinomycetota*, mycobacteria have re-evolved an outer membrane distinct from the Gram-negative outer membrane found in the last bacterial common ancestor. This mycomembrane is composed of waxy mycolic acids and is covalently fused to the arabinogalactan cell wall.

The mycobacterial cell envelope poses a significant barrier to transport of proteins and nutrients in and out of the mycobacterial cell. Therefore, understanding the systems which mycobacteria use for trans-envelope transport is both biologically interesting and important for getting new therapeutic agents inside pathogenic cells. Several genera related to mycobacteria are hypothesised to also have outer membranes based on genetic and biochemical analysis, but most have not yet been thoroughly investigated by cryo-electron microscopy. I will present our group's progress so far in using electron cryotomography to understand the makeup and evolution of *Actinomycetota* cell envelopes and the function of secretion system protein complexes embedded within those envelopes.

HFSP reference number: LT0048/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: HENNELL JAMES, Rory
Host supervisor: MARLOVITS, Thomas

DISENTANGLING MICROBIOME EFFECTS ON PLANT DEVELOPMENT

Alejandra Hernandez Teran*[1]; Maria Rebolleda-Gomez[1]
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Abstract

It's increasingly clear that microbes influence host health, fitness, and even important developmental transitions like metamorphosis in mosquitoes and flowering time. The timing of these transitions is central for organisms responses in changing environments. However, we don't know which microbes alter such traits and how they do it, or whether it's adaptive for the microbes or a consequence of their metabolism. Understanding the role of microbial communities in developmemtal timing of other organisms can help us better predict, and even enhance adaptive responses to changing signals. Using of life-history models and community selection, our goal is to understand microbial effects on plant developmental timing. One intriguing hypothesis is that since microbes are indicators of environmental and ecological conditions, they can provide information about optimal phenology to the plant hosts. We are using *Arabidopsis thaliana* to determine how microbial communities can affect developmental timing. We sampled microbial communities from several environments and inoculated *A. thaliana* seeds to see the effect of those communities on development. Using directed evolution, we serially propagated microbial communities *in planta* and selected communities that promoted extreme phenotypes on developmental timing. After four transfers, we stabilized the community to obtain microbes that reliaby accelarate, delay, or maintain flowering time with respect to uninoculated controls. We are isolating microbial members from the communities with the different effects to inoculate combinations of them and gain insights into potential particular microbes that are behind the developmental effects. In addition, using omics approaches, we are working on the characterization of the community and the metabolic functions that are associated with either late or early flowering, and incorporating our insights into predictive life-history models to evaluate possible effects of microbes on plant adaptation to climate change.

HFSP reference number: LT0047/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: HERNANDEZ-TERAN , Alejandra
Host supervisor: REBOLLEDA-GOMEZ, Maria

MOLECULAR MECHANISM OF PHENOTYPIC MEMORY FOR BACTERIAL GROWTH IN FLUCTUATING ENVIRONMENTS

Edo Kussell*[1]
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Abstract

Bacteria growing in fluctuating environments rely on gene regulatory mechanisms that activate and repress specific genes in response to environmental cues. By measuring their growth in a fluctuating environment using microfluidic devices, we have found that bacteria exhibit phenotypic memory that enables cells to minimize the impact of lag phases on growth. We found that phenotypic memory lasts for multiple generations, and provides a key survival strategy in rapidly fluctuating environments. In this talk, I describe our experiments that reveal the molecular mechanism of phenotypic memory, using synthetic biology tools to engineer a library of strains with different levels of memory. I also highlight our theoretical results that determine which fluctuating conditions select for phenotypic memory, and a biophysical model that enables us to infer the molecular rate constants of the phenotypic memory mechanism from experimental measurements.

Web: www.kussellgroup.org

HFSP reference number: RGY0079/2011
HFSP Award category: ALUMNI Young Investigator Grant
HFSP Award year: 2011
Principal Investigator: KUSSELL, Edo (USA)
Co-Investigators: GUET, Calin (Austria), WAKAMOTO, Yuichi (Japan)

THE REGULATION OF SOCIAL CONFLICT IN THE INDIAN JUMPING ANT (HARPEGNATHOS SALTATOR)

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Abstract

Social insects can undergo dramatic changes in their physiology and behavior to support the collective needs of the colony. In the ant *Harpegnathos saltator*, when a colony loses their queen, workers can transition into reproductive pseudo-queens (gamergates) to re-establish the hierarchy in the colony. In an orphaned colony that lacks queen pheromones, the young workers engage into a dueling behavior by trading strikes with their antennae. Most workers (non-duelers) rapidly abandon the fight and retain their worker status. A few duelers sustain the dueling behavior for months and transit into gamergates to share social dominance in the colony. These gamergates show dramatic physiological and behavioral changes, lay eggs, and cease performing workers' duties. They also have a 5X lifespan extension, and their brain is remodeled and shrinks by 20%. All these transformations are fully reversible when gamergates are placed back in a queened colony and are 'policed' to return to being workers. However, the signals that induces dueling, as well as how the duelers suppress non-duelers from re-engaging in dueling, remain unclear. We hypothesize that uncharacterized queen pheromones repress dueling. In their absence in an orphaned colony, the inhibition is released, and workers begin dueling. The neural circuits responding to the queen pheromones and controlling their own production to regulate dueling and the establishment of queen-like behavior are not known. To identify the queen pheromones, we extracted the cuticular hydrocarbons (CHCs) from both duelers and non-duelers and performed Gas Chromatography-Mass Spectrometry. The CHC profiles between duelers and non-duelers are relatively similar. However, one long-chain hydrocarbon consistently exhibits a stronger signal in the non-duelers, which might self-suppress them from engaging in dueling. Further experiments need to be done to determine the identity of the compound and test its bioactivity. In addition, we are generating transgenic ants that express a calcium indicator (GCaMP6s) in the olfactory sensory neurons to perform functional imaging in response to the queen pheromones. This tool may address whether duelers and non-duelers exhibit different sensitivity to the pheromones, thus sustaining and abandoning dueling, respectively. This work will elucidate the molecular underpinning of social conflict that establishes a new shared hierarchy in ant colonies.

HFSP reference number: LT 0008/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: LEE, Ching-Han
Host supervisor: Desplan, Claude
Reinberg, Danny

MAPPING STRUCTURAL AND FUNCTIONAL CONNECTIVITY OF THE DISTRIBUTED SENSORY SYSTEM IN CHITON ARMOR

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Abstract

Many mollusks produce hard and strong mineral-based exterior shells for protection purposes. While most of these shells are considered "dead" tissue (without any living tissue incorporated), chitons are the only extant mollusks with living tissue embedded within their biomineralized protective shell plates. This innervated tissue, known as aesthetes, forms a complex three-dimensional interconnected network that fills thousands of long, narrow channels running through the shell plates and extending to the shell surface. It has been shown that the aesthetes serve several sensory functions, particularly photoreception. In addition, the aesthetes of certain chiton species include visual elements with different degrees of complexity, ranging from (1) simple photoreceptive organs to (2) eyespots to (3) to image-forming eyes with mineralized lenses that confer spatial vision! This unique feature comprises a 'hard-wired' (embedded in mineralized shells) distributed sensory network (DSN) across the shell plates, contrasting sharply with the visual systems of most other organisms which utilize a small number of sensory organs and a centralized nervous system (e.g., the paired eyes and complex brains of vertebrates). While the current knowledge of chiton DSNs primarily focuses on overall behavioral responses and the fine structures of individual sensory elements, there is limited understanding of their system-level structure and function. How are chiton DSNs wired up? How do they function? How resilient are chiton DSNs to damage? This newly funded HFSP award supports our international, interdisciplinary team of material scientists, biologists, and applied mathematicians in addressing these fundamental questions by establishing the complete 'connectome' of chiton DSNs and investigating their working mechanisms and system resilience. This research aims to transform our current understanding of natural DSNs, with potential impacts on areas such as distributed sensing structures, living materials, and resilient swarm systems. In this poster, we will introduce the general scope and research plans for this project and present ongoing research efforts, such as functional and evolutionary comparison of the chiton DSNs based on shell eyes and eyespots.

HFSP reference number: RGP016/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2023
Principal Investigator: LI, Ling (United of America)
Co-Investigators: BAUM, Daniel (Germany), SPEISER, Daniel (United of America)

CHARTING THE ORIGIN, DIVERSITY AND BIOGEOGRAPHY OF SMALL PROTEINS IN THE GLOBAL OCEAN MICROBIOME

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Abstract

Marine microbes play pivotal roles in various biochemical processes and ecological dynamics within the global ocean microbiome. Among their functional repertoire, small open reading frame-encoded proteins (SEPs) are recognized for their potential involvement in mediating communication and competition. However, the comprehensive understanding of SEPs in ocean microbiomes has been impeded by technical challenges. This project aims to overcome these hurdles by developing a computational framework utilizing machine learning algorithms to identify and characterize SEPs in ocean microbiomes on a large scale. Leveraging recent advancements in metagenomics, such as the reconstruction of metagenome-assembled genomes (MAGs), and considering the substantial phylogenetic and functional diversity inherent in ocean microbiomes driven by complex environmental gradients, this study will explore SEP functional clusters and investigate genetic and environmental factors influencing their evolution. Furthermore, the antimicrobial efficacy of newly discovered SEPs with predicted antimicrobial properties will be experimentally verified. This investigation will be anchored by the Ocean Microbiomics Database (OMD), housing a repository of 12,260 marine geo-referenced samples. These samples have yielded over 250,000 genomes, subsequently de-replicated into more than 40,000 prokaryotic species-level clusters. Beyond providing rich annotations and sample metadata, the OMD utilizes contemporary bioinformatic tools to offer genome-scale predictions, aiding in the discovery of novel taxa and genes, including the identification of SEPs. This integrated approach holds promise for advancing our understanding of small protein diversity and biogeography in ocean microbiomes. It is poised to shed light on intricate microbial community interactions and to inspire innovative biotechnological applications.

HFSP reference number: HFSP LT0050/2023
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: Miravet-Verde, Samuel
Host supervisor: Sunagawa, Shinichi

HFSP-240019

A COMPREHENSIVE MATHEMATICAL FRAMEWORK FOR COMPUTATIONAL BIOLOGY: DYNAMICAL GRAPH GRAMMARS FOR CYTOSKELETON IN PLANT CELLS AND IN SYNAPSES

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Abstract

The mathematical frameworks of computational science include modeling spatially continuous fields with partial differential equations, and modeling particles with ordinary or stochastic differential equations together with some provision for particle interaction, for example by way of fields or collision events, among others. When changing from one spatiotemporal scale to another, modelers must often switch frameworks. Starting from stochastic chemical kinetics we have elaborated another and unifying approach. There are many *topological* systems in biology such as the whole-organism structure of plants, dominated by branching, or neuronal architecture or finer-scale cytoskeleton, that don't fit naturally into particle or field paradigms. Instead they fit into alternative grammar-like dynamical frameworks similar to Lindenmayer systems but augmented with quantitative dynamics in continuous time. Thus arise Dynamical Graph Grammars (DGGs), that is, grammars that specify models that describe dynamic graphs [1].

In a DGG, each rewrite rule represents a constituent biological process, local in the graph (where a graph is a set of parameter-bearing nodes connected by purely topological edges), together with a graph-local dynamics expressed as structure-changing rewrite rules. Such rules can directly change the number and nature of objects present, and their graph connectivity relationships, and/or the values of their parameters, either in discrete events or continuously in time. Ordinary and stochastic differential equations can be subsumed into particular rules, so DGGs don't lose generality compared to ODEs. PDEs can be approximated on "stencils" in adaptive grid graphs by DGGs as discussed in reference [1], or by graph limits [2], or accommodated by nodes bearing functions that obey PDEs.

We have developed models for cortical microtubule arrays in plant cells, and actin cytoskeleton in a dendritic spine head, expressed in terms of DGGs. We are building a new simulator for a substantial and much-needed speed improvement for spatially local DGGs, that will enable multiscale modeling using the generality of DGGs to stay within the paradigm at multiple scales. These efforts may help to bring to fruition the promise of dynamical graph grammars in biological modeling.

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HFSP reference number: RGP0023/2018
HFSP Award category: ALUMNI Research Grant
HFSP Award year: 2018
Principal Investigator: HAMANT, Olivier (France)
Co-Investigators: DUMAIS, Jacques (Canada), MJOLSNESS, Eric (USA), SCHNITTINGER, Arp (Germany)

THE ROLE OF HOST-ODOR SENSING IN THE EVOLUTION OF MOSQUITO VECTOR COMPETENCY: A FRIEND OR A FOE

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Abstract

Our past work has alluded to environmental odor-sensing as a route to immune-priming and competency. We found that preconditioning *Drosophila* larvae to pathogenic odors led to development of a robust immune system such that, when infected, these animals responded to infections much superiorly than un-exposed animals. While the results implied olfaction-based control of immune potential, in this recently funded HFSP research project we aim at exploring the sense of smell in mosquitoes and how their odor-based anthropophilic behavioral adaptation has impacted their ability to carry and transmit infections. Our preliminary findings in *Anopheles stephensi*, a major vector for malaria, alludes to a dramatic influence of environmental volatile-sensing on their *Plasmodium* infection rates and consequently vector potential. In particular, we find that acetophenone and octanal, which are distinct volatiles that are predominantly produced by the host, and stimulates mosquito olfaction for attractiveness, their exposure and sensing is linked to improved *P.berghei* infection clearance. As result when tested for their ability to transmit infection, they performed poorly as vectors. Although the data prove the influence of odor-detection on sensitivity of *Anopheles* to infection, the distinct influence of host-odor sensing and development of stronger immune-potential is unexpected. Going forward, we will explore the mechanistic underpinnings of this sensory/immune cross-talk, but importantly, how host-specific volatiles, especially human specific odors impact mosquito immunity and infection susceptibility will be discerned. We predict that anthropophilic behavior in mosquitoes which is defined by specific volatiles, may play a key role in defining mosquito receptivity to infections. Similar work led by our other team member shows susceptibility of *Aedes* to viral infection upon blocking olfactory sensing. Although in its preliminary stages, the data indicates a deeper physiological connection of the mosquito sensory system beyond odor-sensing. We believe that the findings will yield fundamental insights into how changes in sensory physiology have led to the evolution of vector competence.

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HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2023
Principal Investigator: **Tina Mukherjee (India)**
Co-Investigators: **Joao Marques (France), Benjamin Matthews (Canada), Mario Recker (UK)**

REGULATION OF CELL IDENTITY AND IDENTITY BREAKERS

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Abstract

Maintenance of cell identity requires active supervision, and failure to maintain the differentiated state results in disease such as cancer. Identity Breakers, (IBs), are genes that unlock cell identity, enhance cellular reprograming and initiate or drive cancer. However, the identity of IBs, the mechanisms by which they act and their role in tumorigenesis are largely unknown. A powerful model for studying cell identity is the *Drosophila* gut epithelia. Focusing on differentiated enterocytes (ECs) we previously discovered the core gene-framework and mechanisms that safeguard ECs identity (1,2). Currently, focusing on post-transcriptional regulation of cell identitiy, and using single-cell RNA-seq combined with G-TRACE lineage tracing we discovered a stem cell-related translational repression machinery that ectopically accumulates during aging, breaks EC identity, disrupts tissue homeostasis. We observed that conditional expression of these genes in young ECs unlock cell identity and their elimination form aged ECs restore EC identity and suppresses aging phenotypes and extend longevity. Moreover, we will present data regarding a ubiquitin machinery involved in the active regulation of identity breakers and that is required for maintaining EC identity.

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2. Erez, N., Israitel L., Bitman-Lotan E., Wong, W. H., Raz G., Danial S., Flint Brodsly N., Belova, E., Maksimenko, O., GeorgievP., Druley, T., Mohan R., Orian A. (2021) A Non-stop identity complex (NIC) supervises enterocyte identity and protects from pre-mature aging. *e-Life* 10:e62312.

Web: https://orian.net.technion.ac.il/

HFSP reference number: HFSP-240054
HFSP Award category: ALUMNI Career Development Award
HFSP Award year: 2005

COEVOLUTION OF BODY MORPHOLOGY, NEURAL CIRCUITS, AND BEHAVIOR

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Abstract

For different species to become adapted to their environments, evolution often reshapes an animal's body morphology as well as its neural circuitry, enabling behaviors required to flourish in specific ecological niches. The wide array of body types across the animal kingdom is clear to see, but it is less well understood how different species' nervous systems have changed to control these various bodies. To understand the coevolution of body morphology, neural circuits, and behavior, we are studying two species of *Drosophila* with a striking morphological difference – male *Drosophila prolongata* have forelegs that are disproportionately enlarged and elongated compared to both females of their own species and to the commonly studied fruit fly *Drosophila melanogaster*. These males' long forelegs play a central role in courting females, but how the leg growth has influenced other behaviors like walking, and how the nervous system has co-evolved to adapt to the large legs, remain open questions. The genetic tractability of *Drosophila* species makes this an excellent opportunity to dissect this topic in detail. Specifically, we are investigating a few questions:
1) Which joints in the enlarged legs show different movement patterns to maintain functional behaviors like walking and grooming?
2) Has the region of the nervous system that controls the enlarged forelegs expanded in male *D prolongata*? Alternatively, could adaptation to the leg musculature be sufficient to compensate for the legs' size?
3) How have activity patterns in the nervous system's motor control circuits had to change?

We have been developing setups for comparing the limb anatomy, behaviors, and nervous systems of *D prolongata* and *D melanogaster*. Specifically, we use high speed videography and deep-learning-based limb tracking algorithms to describe the exact movements that each species makes, allowing us to detect adaptations to walking and other behaviors driven by the disproportionate limb enlargement. Second, we use transgenic *D prolongata* expressing fluorescent proteins to investigate structural changes in the part of the nervous system controlling the enlarged forelegs. Finally, we are developing a light sheet microscope capable of recording neural activity from the motor circuits of behaving flies of both species. Together these approaches will reveal how evolution orchestrates multiple types of adaptations to give rise to well-adapted species.

HFSP reference number: LT0029/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: PHELPS, Jasper
Host supervisor: RAMDYA, Pavan

ANT AGGREGATION PHEROMONES: FROM SOCIAL BEHAVIOR TO NEURAL CODING

Matteo Rossi*[1]; Tiphaine Bailly[1]; Erik Frank[2]; Jocelyn Millar[3]; Dennis Mendelez[1]; Dominic Frank[1]; Thomas Schmitt[2]; Daniel Kronauer[1]

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Abstract

The capacity to form groups is at the core of insect societies. However, how this ability is encoded in their sensory and neural systems is unknown. A pheromone (odor cue) can be expected to mediate aggregation in ants, similarly to other aggregative behaviors in insects, but this sensory cue has not yet been described in any ant species. Here, we provide evidence that there is a low-volatility odor cue that mediates nest aggregation in the clonal raider ant (*Ooceraea biroi)*. We provide evidence that this cue is not a learned odor cue but a fixed (species-specific) cue that elicits nest formation across genetically distinct *O. biroi* populations. Furthermore, we show that this odor cue elicits nest-related behavioral tendencies. We describe our results to pinpoint the chemical basis of this aggregation cue. Finally, we describe our recent efforts to establish a contact-based calcium-imaging set-up to study how perception of this pheromone and related behaviors are encoded in olfactory sensory neurons, using an established transgenic line. Overall, *O. biroi*, with its genetic amenability and evidence of an aggregation pheromone, presents an exciting system to gain a mechanistic understanding of how sensory perception can sustain group formation and social behaviors in animal societies.

HFSP reference number: LT0023/2023
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: ROSSI, Matteo
Host supervisor: KRONAUER, Daniel

INHIBITING DENGUE VIRUS INFECTION: ROLE OF 25-HYDROXYCHOLESTEROL IN MEMBRANE FUSION DYNAMICS

Debayani Chakraborty[1]; Achinta Sannigrahi[1]; Haneefa Muhsin[1]; Rahul Roy*[1]
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Abstract

Dengue virus, and other viral pathogens such as SARS-CoV-2 and Zika, utilize specialized glycoproteins to facilitate the fusion of its lipid membrane with that of the host cell, a critical step for viral entry and infection. This membrane fusion process is significantly influenced by factors such as lipid composition, membrane architecture, and embedded receptors; however, the specific mechanisms governing dengue virus membrane fusion are not fully elucidated. Our research focuses on dissecting these mechanisms using both bulk and single-virus fusion assays, and exploring how lipid composition affects these dynamics.

We demonstrate that 25-hydroxycholesterol (25-HC), an oxidized form of cholesterol, significantly impedes the membrane fusion capability of the dengue virus, thereby inhibiting viral entry and infection in cell cultures. This inhibitory effect of 25-HC is further supported by our observations that the CH25H gene, encoding cholesterol 25-hydroxylase, is upregulated in various cell types as a defense response to viral infection. Pre-exposure to 25-HC not only upregulates CH25H but predominantly acts by inhibiting viral membrane fusion.

These insights into the modulation of dengue virus membrane fusion by lipid cofactors in the host cell membrane present a promising avenue for the development of strategies aimed at inhibiting viral membrane fusion, thereby potentially curtailing the spread of the infection. For example, we show that other virus entry and replication inhibitors display strong positive synergy in virus inhibition with 25-HC. This study underscores the potential of targeting lipid-mediated processes as a therapeutic strategy against dengue and possibly other similar viral infections.

HFSP reference number: RGP0047/2020
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2020
Principal Investigator: ROY, Rahul (INDIA)
Co-Investigators: HOWORKA, Stefan (UK), AKSIMENTIEV, Aleksei (USA)

FANZOR IS A EUKARYOTIC PROGRAMMABLE RNA-GUIDED ENDONUCLEASE

Makoto Saito*[1]; Peiyu Xu[1]; Guilhem Faure[1]; Samantha Maguire[1]; Soumya Kannan[1]; Han Altae-Tran[1]; Sam Vo[1]; Anan Desimone[1]; Rhiannon K. Macrae[1]; Feng Zhang[1]
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Abstract

RNA-guided systems, which use complementarity between a guide RNA and target nucleic acid sequences for recognition of genetic elements, have a central role in biological processes in both prokaryotes and eukaryotes. For example, the prokaryotic CRISPR–Cas systems provide adaptive immunity for bacteria and archaea against foreign genetic elements. Cas effectors such as Cas9 and Cas12 perform guide-RNA-dependent DNA cleavage. Although a few eukaryotic RNA-guided systems have been studied, including RNA interference and ribosomal RNA modification, it remains unclear whether eukaryotes have RNA-guided endonucleases.

Recently, a new class of prokaryotic RNA-guided systems (termed OMEGA) was reported. The OMEGA effector TnpB is the putative ancestor of Cas12 and has RNA-guided endonuclease activity. TnpB may also be the ancestor of the eukaryotic transposon-encoded Fanzor (Fz) proteins, raising the possibility that eukaryotes are also equipped with CRISPR–Cas or OMEGA-like programmable RNA-guided endonucleases.

Here we report the biochemical characterization of Fz, showing that it is an RNA-guided DNA endonuclease. We also show that Fz can be reprogrammed for human genome engineering applications. Finally, we resolve the structure of *Spizellomyces punctatus* Fz at 2.7 Å using cryogenic electron microscopy, showing the conservation of core regions among Fz, TnpB and Cas12, despite diverse cognate RNA structures. Our results show that Fz is a eukaryotic OMEGA system, demonstrating that RNA-guided endonucleases are present in all three domains of life.

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HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2020
Fellow: Saito, Makoto
Host supervisor: Zhang, Feng

MAPPING THE BIOSYNTHESIS OF BMP, A KEY PHOSPHOLIPID ENABLING LIPID DEGRADATION IN THE LYSOSOMES

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Abstract

Bis(monoacylglycero)phosphates (BMP) is a negatively charged phospholipid exclusively localized in the lysosomes, where it helps lysosomal lipases dock at intralumenal vesicles (ILVs) and degrade membrane lipids. Altered BMP levels result in lysosomal lipid accumulation and may promote neurodegeneration. In the lysosome, BMP needs to be protected from degradation by lipases that it interacts with. This protection is achieved by the unique stereochemistry of BMP: its two glycerol moieties are in the *S,S* stereo-conformation, unlike other phospholipids that have glycerol in the *R*-stereoconformation. How the crucial *S,S* form of BMP is synthesized has been a mystery for decades. Here we identify PLD3 and PLD4 as the enzymes that synthesize *S,S*-BMP in the lysosomes. We found, by overexpression in cells and with purified enzymes, that PLD3 and PLD4 utilize *R,S*-lysophosphatidylglycerol (lyso-PG) and *racemic*-monoacylglycerol as substrates to catalyze a transphosphatidylation reaction that synthesizes *S,S*-BMP. The transphosphatidylation reaction enables the crucial stereo-conformational change in a glycerol moiety. In cells and murine models, deletion of PLD3 or PLD4 markedly reduced BMP levels, leading to ganglioside accumulation and lysosomal abnormalities. Notably, pathological mutations of PLD3 associated with neurodegeneration (V232M for Alzheimer's disease risk, and L308P causing spinocerebellar ataxia) exhibited reduced BMP synthesis activities. We conclude that PLD3 and PLD4 are the crucial lysosomal enzymes mediating *S,S*-BMP synthesis. Our studies solve a key problem in lysosomal biology and shed new light on lipid metabolism-related mechanisms that promote neurodegenerative disease.

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HFSP reference number: LT0025/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: Singh, Shubham
Host supervisor: Farese, Robert V. and Walther, Tobias C.

EXPLORING MORPHO-FUNCTIONAL RELATIONSHIPS IN CEPHALOPOD BEAKS: INSIGHTS FROM EXPERIMENTAL AND COMPUTATIONAL ANALYSIS

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Abstract

Cephalopods are marine molluscs, including octopus, squid, and cuttlefish. Carnivorous predators with more than 800 species living in all marine ecosystems, they exhibit a wide range of feeding behaviours and foraging strategies related to their lifestyle, habitat and morphological adaptations. Octopod species (octopus) usually feed on hard preys including crustaceans and molluscs, while decapod species (squids and cuttlefishes) mostly eat fish. They all feed using a pair of chitinous beak, which plays a crucial role in capturing, biting and tearing apart their prey. The size and shape of the beak vary extensively between species. If these beaks are widely used for taxonomy, recent study shows that their morphology is not only driven by phylogeny, but also carries an ecological signal and may therefore reflect adaptation to specialized diets.

The aim of this study is to examine the relationship between the shape of cephalopod beak rostra and their function, using a combination of experimental and computational methods. Fourteen rostrum models representing a range of beak morphologies were 3D-printed and subjected to uniaxial puncture tests to assess puncture ability. Finite Element Analysis (FEA) was used to investigate the relationship between form and function under loading conditions simulating biting and pulling, analyzing stress distribution across different rostra.

Our findings show that rostrum size significantly impacts puncture performance, with smaller rostra requiring less force and displacement to pierce a given target. However, larger rostra exhibit higher structural stiffness, making them more susceptible to stress during biting. Species-specific differences in puncture abilities were observed in morphology-driven tests, with rostrum sharpness playing a crucial role. FEA results suggest that longer and sharper rostra may be more prone to stress, potentially affecting overall structural integrity. These results highlight the trade-off between rostrum size and sharpness in cephalopod beaks, with implications for prey selection and feeding efficiency. The study enhances understanding of the morpho-functional aspects of cephalopod beaks and their role in prey capture and consumption, providing insights into the evolutionary pressures shaping these remarkable marine predators.

HFSP reference number: LT000476/2021-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2021
Fellow: SOUQUET, Louise
Host supervisor: MOAZEN, Mehran

INTEGRATING PLANT GENETICS AND BEE FORAGING BEHAVIOUR TO UNDERSTAND THE CO-EVOLUTION OF PLANT-POLLINATOR INTERACTIONS

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Abstract

Plant-pollinator interactions represent a core mutualism that supports terrestrial ecosystems and are vital for human agricultural production and food security. While it is thought that nutritional values of floral rewards are a primary driver of pollinator attraction, floral traits are co-evolved in wild plant species and thus it is difficult to test this hypothesis directly. By crossing a self-fertilizing *Capsella rubella* (*Cr*) and an outcrossing, pollinator dependent species *C. grandiflora (Cg)*, we created recombinant inbred lines (RILs) that vary across floral traits. Here, we developed highly controlled experimental system to evaluate the mechanisms underlying foraging preferences of three species of bees (*Apis mellifera*, *Osmia cornifrons* and *Megachile rotundata*), to two parental and 16 *Capsella* RILs. In laboratory arenas and outdoor flight cages, we continuously recorded bee foraging behavior to determine if different floral traits (flower abundance, petal size, macronutrient content) influenced visitation patterns. While all bee species were more attracted to inflorescences based on floral morphology (either more flowers or petal size), only *M. rotundata* visits were strongly associated with pollen nutritional quality, with preference to plants with higher pollen protein to lipid content. These studies demonstrate that, although all three bee species collect pollen as their sole source of protein and lipids for themselves and their offspring, only one appears to differentiate among flowers based on these pollen nutrients. How these different foraging strategies evolved, and influence plant-pollinator ecological networks remains to be determined. We further studying species-specific macronutrient regulation in bees using synthetic diets. Complementing these specific macronutrient requirements and regulation in bees with their foraging behavior in the field help us to explain the patterns of host-plant species choice among bees, enabling the creation of habitats that are nutritionally and ecologically beneficial for a diverse community of pollinators.

HFSP reference number: RGP0057/2021
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2021
Principal Investigator: SICARD, Adrien (Sweden)
Co-Investigators: RISSE, Benjamin (Germany), GROZINGER, Christina (USA)

EXPLORING NEURAL CREST CELL DYNAMICS IN NEWT LIMB REGENERATION

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Abstract

Neural crest cells arise during neurulation in the closing neural tube that will form the central nervous system (CNS). They subsequently migrate away from the dorsal aspect of the neural tube, navigating throughout the periphery where they differentiate into diverse cell types in numerous tissues and organs. While neural crest cells are well-known to be essential for normal development it has recently been shown that they are involved in organ regeneration during adult heart regeneration in zebrafish and digit tip regeneration in mice. This raises the intriguing possibility that neural crest cells may contribute to and/or be essential in other regenerative systems. To test the hypothesis, I have employed *Pleurodeles waltl* (Iberian ribbed newt) as an emerging model organism for regenerative biology. I have used reverse genetics to generate several transgenic lines to genetically label and trace neural crest-derived cells *in vivo* during regeneration. By analyzing of newt limb regeneration as a function of time, I observed a large population of cells expressing the well-known neural crest marker *Sox10* invading the middle of regenerating blastema. They appear transiently when the blastema cells are most proliferative and in a de-differentiated state. Combining high-sensitivity staining with tissue-specific markers, light-sheet 3D imaging, and genetical tools that include Sox10 transgenic newts and targeted gene knockout *in vivo*, I will discuss the characters and their function of neural crest cells in newt limb regeneration.

HFSP reference number: LT0009/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: SUZUKI, Miyuki
Host supervisor: BRONNER, Marianne E.

DANCING MOLECULES WITH SUB-NANOMETER STEPS

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Abstract

Chemists have long sought to observe and control the motion of molecules at the nanoscale. To this end, recent years have witnessed the maturation of the field of 'molecular machines', inspired by the mechanics of engineered objects. However, many efforts thus far have included rather slow and kinetically demanding chemical reactions, hampering the progress in real-world applications of such nanomachines. Here, harnessing the chemical reactivity of selenium – an element just below sulfur in the periodic table – we present our work-in-progress towards rapid and controlled motion of molecules under nanopore confinement. The single-molecule reactivity profiles and individual chemical stepping were characterized within an alpha-hemolysin nanopore, at millisecond resolution. The reversible chemical reactions presented here can be versatile, enabling more complex activities such as directed rotational motion on an axis, towards non-chemically-fuelled protein motors; and unidirectional movement of biopolymers along a defined track, towards single-molecule sequencing of proteins. Moreover, the chemistry of selenium can be utilized to construct dynamic chemical reaction networks, aiding in our understanding of biological redox reactions and how out-of-equilibrium systems might have emerged from organic chemistry.

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HFSP reference number: LT 0040/2022-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2022
Fellow: TOPARLAK, Duhan
Host supervisor: BAYLEY, Hagan

OPRF IMPACTS PSEUDOMONAS AERUGINOSA BIOFILM MATRIX EDNA LEVELS IN A NUTRIENT-DEPENDENT MANNER

Erin K. Cassin[1]; Sophia A. Araujo-Hernandez[1]; Dena S. Baughn[1]; Melissa C. Londono[1]; Daniela Q. Rodriguez[1]; Natalie S. Al-Otaibi[2]; Aude Picard[1]; Julien Bergeron[3]; Boo Shan Tseng*[1]

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Abstract

Biofilms are microbial aggregates held together by a self-produced extracellular matrix crucial to the increased resilience of resident cells against external stressors. The biofilm matrix is composed of exopolysaccharides, extracellular nucleic acids (eDNA and eRNA), membrane vesicles, and proteins. While proteomic analyses have identified numerous matrix proteins, their functions in the biofilm remain understudied compared to the other biofilm components. In the *Pseudomonas aeruginosa* biofilm, several studies have identified OprF (an OmpA-family outer membrane porin) as an abundant matrix protein and, more specifically, as a component of biofilm membrane vesicles. However, current data describing the effects of OprF in the *P. aeruginosa* biofilm is limited. Here we identify a nutrient-dependent effect of OprF in static biofilms, whereby Δ*oprF* cells form significantly less biofilm than wild type when grown in media containing glucose or low sodium chloride concentrations. Interestingly, this biofilm defect occurs during late static biofilm formation and is not dependent on the production of PQS, which is responsible for outer membrane vesicle production. Furthermore, while biofilms lacking OprF contain approximately 60% less total biomass than those of wild type, the number of cells in these two biofilms is equivalent. We demonstrate that *P. aeruginosa* Δ*oprF* biofilms with reduced biofilm biomass contain less eDNA than wild-type biofilms. These results suggest that the nutrient-dependent effect of OprF is involved in the maintenance of mature *P. aeruginosa* biofilms by retaining eDNA in the matrix. Since eDNA is necessary for *P. aeruginosa* biofilm structure formation, targeting OprF may be an effective anti-biofilm strategy, as loss of this protein results in the degradation of the protective matrix.

HFSP reference number: RGY0080/2021
HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2021
Principal Investigator: DURHAM, William (UK)
Co-Investigators: WHITNEY, John (Canada), TSENG, Boo Shan (USA), BERGERON, Julien (UK)

POSTER SESSION

THE PYRUVATE DEHYDROGENASE COMPLEX REGULATES MITOCHONDRIAL MATRIX PROTEIN PHOSPHORYLATION AND MITOPHAGIC SELECTIVITY, INDEPENDENT OF ITS CATALYTIC ACTIVITY

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Abstract
Mitophagy, or the autophagic degradation of mitochondria, is an important housekeeping function of eukaryotic cells that prevents the accumulation of defective mitochondria due to oxidative damage and spontaneous mutations. The culling of defective mitochondria is thought to delay the onset of aging symptoms, and defects in mitophagy have been linked to late onset disorders such as Parkinson's disease and type II diabetes. We previously demonstrated that different mitochondrial matrix proteins undergo mitophagy at different rates, and that mitochondrial matrix protein phosphorylation and dephosphorylation can generate a segregation principle that would couple with mitochondrial fission and fusion dynamics to selectively degrade sub-sets of mitochondrial proteins. We now demonstrate a role for the pyruvate dehydrogenase complex (PDC) as a signaling nexus in this regulatory network. We demonstrate that the PDC controls the activities of its cognate kinases and phosphatases towards other mitochondrial matrix proteins, and that this novel function can be uncoupled from the pyruvate dehydrogenase catalytic function itself. Our data support a model where the PDC functions in a structural role to allosterically regulate its associated kinases and phosphatases towards 'third party' proteins in the mitochondrial matrix, suggesting a possible regulatory link between the levels of central mitochondrial metabolites and the regulation of mitophagic selectivity.

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HFSP reference number: LT00040/1996-M
HFSP Award category: Other
HFSP Award year: 1996
HFSP long term fellowship,(Award year 1996)

BIOSENSING OF DISEASE ENVIRONMENTS FOR RATIONAL DESIGN OF PRO-DRUGS

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Abstract

Cancer is characterized by dysregulated extracellular protease activity, which presents an opportunity to design precise cancer diagnostics and therapies (e.g. prodrugs). These molecules typically consist of a drug moiety or a reporter molecule, a peptide linker with a proteolysis substrate sequence, and a masking domain. When delivered to the tumor, the elevated proteolysis at its extracellular matrix (ECM) cleaves the substrate, removing the masking domain and activating the drug or reporter. However, the design of these molecules requires a comprehensive understanding of the proteolytic landscape in tumors, which is currently lacking. Our understanding of proteolysis across different tumor tissues, types, and stages, as well as in healthy tissues, remains incomplete, hindering the development of precise protease-based diagnostics and prodrugs. To address this knowledge gap, we herein present a comprehensive peptide library displayed on yeast, which facilitates multiplexed and detailed profiling of recombinant proteases and proteolysis substrates in tumor and healthy tissues ECM samples.

HFSP reference number: LT0019/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: ALGOV, Itay
Host supervisor: ZHOU, Xin

CONTROLLING MACROSCALE MORPHOLOGY IN DNA-BASED ASSEMBLY USING ACOUSTIC ENERGY

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Abstract

One of the main challenges of developing bottom-up designed materials is the issue of scaling their formation and shaping them into a desired morphology. A high degree of nanoscale control hinders the ability to form nanomaterials with predefined macroscale morphology. DNA nanotechnology allows accurate spatial control at the nanoscale which enables fabrication of intricate organizations; yet, structural arrangement at the macroscale remains a challenge. We developed an assembly approach driven by acoustic waves in order to control the morphology of DNA-assembled materials at the scales from tens of microns to millimeters, thus complementing a nanoscale assembly regime offered by DNA-guided methods. Specifically, we explored the use of standing surface acoustic waves (SSAW) to direct assembly and control morphology of DNA origami based crystal lattices. By controlling both acoustic forces and temperature, we investigated the assembly process at different scales by a combination of optical microscopy, small-angle x-ray scattering and electron microscopy techniques. We further studied the nucleation, crystal fusion and growth under different acoustic conditions. The developed approach allows to form macroscale nanomaterials with prescribed morphology, as defined by the acoustic field, while their nanoscale organization is programmed by DNA. Our experimental observations are supported by a model that incorporates nucleation dynamics, diffusion-limited growth, and the effects of acoustic driving. The model provided valuable insights into the impact of acoustic waves on suppressed nucleation and crystal growth. Overall, our study demonstrates the potential of acoustic waves as a complementary method for controlling the morphology of DNA-assembled nanomaterials at the macroscale. This approach expands the scope of DNA nanotechnology and paves the way for the fabrication of nanomaterials with tailored properties and functionalities for a wide range of applications.

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HFSP reference number: LT000158/2021-C
HFSP Award category: ALUMNI Cross-disciplinary Fellowship
HFSP Award year: 2021
Fellow: ARNON, Zohar
Host supervisor: GANG, Oleg

CHARTING THE SYMBIOSIS SEASCAPE: EXPLORING VARIATION IN MARINE MICROBIAL SYMBIOSES WITH COMPARATIVE AND POPULATION GENOMICS

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Abstract

For host organisms that are dependent on microbial symbionts, symbiont variation within and between populations has the potential to influence both ecological and evolutionary processes. Yet, for most marine microbial symbioses, population- and individual-level symbiont variation is poorly understood. Here, I present population and comparative genomics work from marine symbiotic associations regarding strain-level variation within and across host species. We have found that symbiont transmission mode, habitat characteristics, and/or ecological traits impact local adaptation and geographic population structure. Altogether, this work contributes to our understanding of how neutral and adaptive processes shape the ecology and evolution of microbial symbioses.

Web: www.beinartlab.com

HFSP reference number: RGEC29/2024
HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2024
Principal Investigator: Husnik, Filip (Japan)
Co-Investigators: Beinart, Roxanne (USA), Stairs, Courtney (Sweden)

THE RIBOSOME LOWERS THE ENTROPIC BARRIER OF PROTEIN FOLDING

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Abstract

Most proteins fold co-translationally during biosynthesis on the ribosome and co-translational folding energetics, pathways, and outcomes of many proteins have been found to differ considerably from those in refolding studies. The origin of this folding modulation by the ribosome has remained elusive. We have determined structures of the unfolded state on and off the ribosome, which reveal that the ribosome entropically destabilises the unfolded nascent chain. Quantitative 19F-NMR shows that this destabilisation reduces the entropic penalty of folding by ca. 30.kcal.mol-1 promoting formation of partially-folded intermediates on the ribosome an observation that extends to other protein domains. These entropic effects result in the ribosome protecting the nascent chain from mutation-induced unfolding, suggesting a crucial role of the ribosome in supporting protein evolution. Our findings establish the physical basis of the distinct thermodynamics of co-translational folding

Recent relevant group references

i) The ribosome lowers the entropic penalty of protein folding Streit JA...Christodoulou, J*. in review..Nature (2024); ii) The ribosome stabilises partially folded intermediates of a nascent multi-domain protein Chan SHS..Christodoulou, J*. Nat.Chem. (2022); iii) Modulating co-translational protein folding by rational design and ribosome engineering Ahn M... Christodoulou, J. Nat.Comm. (2021); iv) Folding competes with interactions between the disordered nascent chain and the ribosome Cassaignau, AME..Christodoulou, J*.,Nat.Chem.(2021); v) Common sequence motifs of nascent chains engage the ribosome surface and trigger factor Deckert, A...Christodoulou, J.* PNAS (2021); vi) Nascent chains can form co-translational folding intermediates that promote post-translational folding outcomes in a disease protein. Plessa E... Christodoulou, J.* Nat.Comm. (2021); vii) Nascent chain dynamics and ribosome interactions within folded ribosome-nascent chain complexes observed by NMR spectroscopy Burridge C... Christodoulou, J.* Chem Sci. 12(39):13120-13126 (2021) viii) How does the ribosome fold the proteome? Cassaignau, AME, Cabrita, LD, Christodoulou, J.* Annual Reviews of Biochemistry (2020); ix) Nature and Regulation of Protein Folding on the Ribosome. Waudby CA.,..Christodoulou, J.* TiBS, (2019)

HFSP reference number: RGY0067/2007-C
HFSP Award category: ALUMNI Young Investigator Grant
HFSP Award year: 2007

THE HEART ADAPTS TO VIRAL INFECTION IN ONE OF THREE WAYS

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Abstract

Background: There are multiple viruses that infect the human heart and cause inflammation called myocarditis. Patients with viral myocarditis have an equal chance of i) stabilizing, ii) recovering spontaneously, or iii) progressing in disease severity. Due to patient risk and a lack of treatment options, human hearts are rarely tested for viruses. As a result, it is not well understood how human hearts respond to long-term viral infection on a cellular level.

Methods: Unmapped RNA-sequencing reads from 979 publicly available human heart samples were used to detect undiagnosed heart infections. Consensus clustering and gene set enrichment analysis were used to identify patterns of host gene expression in the virus-positive samples. In parallel, twelve clonal cardiac cell lines were engineered to express chronic levels of coxsackievirus B3 (a known cardio-pathogenic virus) and were treated with inhibitors and activators of the NFκB and p38–MK2 signaling pathways, followed by RNA-sequencing.

Results: We detected cardio-pathogenic viruses in 19.3% of the human-derived samples. These virus-positive samples showed one of three robust adaptations: increased NFκB-associated inflammation, increased AU-rich elements (mRNA instability motif regulated by p38–MK2), or decreased AU-rich elements. In the inflammatory group, genes associated with heart failure were also upregulated, while the AU-rich element high group showed signs of resolved infection. The three adaptations were not specific to any viral species. Furthermore, the same three adaptations were observed in the twelve clonal cardiac cell lines. Inhibiting the p38–MK2 or NFκB pathways individually caused the clones to shift to the decreased AU-rich element state, implying that this adaptation is a signaling ground state. To test if the adaptations are a general response to cardiac infection, we compared the adaptations to known cardio-pathogenic viral infections in human and animal hearts and we observed all three adaptations recur in the independent datasets.

Conclusions: Nearly one in five hearts examined had evidence of cardio-pathogenic viral infection, which is a substantial proportion of the population. Furthermore, we find that the virally infected hearts adapt in one of three interconnected ways, which may have different clinical prognoses or responses to treatment. This knowledge provides a framework for interpreting viral heart infections and may lead to interventions that halt the progression to heart failure.

HFSP reference number: LT000469/2021-L
HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2021
Fellow: GRIFFITHS, Cameron
Host supervisor: JANES, Kevin

ASSOCIATION OF HLA CLASS II ALLELES IN CHILDREN AND YOUNG ADULTS WITH ACUTE RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE FROM LIMPOPO PROVINCE, SOUTH AFRICA

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Abstract

Introduction: Acute rheumatic fever (ARF) leading to rheumatic heart disease (RHD) is an immunological complication of Group A Streptococcus[1][2][3]. The conditions continue to affect children and young adults mostly in poverty-stricken populations. Reports have shown an association between human leukocyte antigens (HLA) alleles and the risk of developing ARF/RHD [3]. The current study aimed to describe the epidemiology and genomic HLA Class II profiles of patients with ARF and/or RHD in Limpopo Province, South Africa.

Methodology: A retrospective assessment of the Limpopo province RHD patient registry was conducted to determine the RHD prevalence rate. Additionally, blood samples for HLA typing were collected from patients aged 3, having ARF (50) and RHD (50) at two regional and provincial hospitals of Limpopo province. Next-generation sequencing of the HLA Class I and Class II types 11 loci platform was performed.

Results: The recorded RHD prevalence rate was 21,7%. The mean age of the patients was 11 years. There was a distribution of 55% males to 45% females. HLA Class II DRB1*11 (96% and 100%), DRB1*06 (72% and 98%), and DRB1*07 (46% and 62%) were the most common alleles in ARF and RHD patients respectively. These 3 HLADR allelic types (HLADR6, DR7, and DR11) were found significantly ($p<0.05$) more in both groups.

Conclusions: The results of this baseline study for HLA types in the selected group of patients may support the hypothesis that certain HLA class II alleles are associated with the risk of RHD. Black South African communities in Limpopo province are harboring HLADR1 and DR6 alleles that may be contributing towards RHD susceptibility. The study is ongoing.

PROBING ULTRAFAST DYNAMICS IN ARTIFICIAL LIGHT-HARVESTING SYSTEMS

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Abstract

Photosynthetic organisms rely on light to synthesize important chemical feedstocks. On a molecular level, the flow of protons and electrons after absorption of a photon is controlled by sophisticated nano-machinery. Through this complex dance, biological systems power most life on Earth. While there is vast knowledge on how nature uses light to fuel life, true understanding is proven by successful replication of the process.

The long-term goal of this project is the creation of synthetic nano-machinery with dynamic control over the excitons for energy capture and conversion. The creation of such a light-harvesting system advances our understanding of this fundamental biological process while simultaneously introducing a new energy material for next-generation bioinspired devices. As the key processes occur on ultrafast time scales, time-resolved optical spectroscopy with femtosecond resolution will be used to track the different species via their distinct absorption features.

As a first step in this project, we present an optimized laser spectroscopic setup to investigate ultrafast light-initiated dynamics on a femtosecond timescale. We detail the different enhancements such as pulse compression using prisms as well as second harmonic generation. The viability of the custom spectrometer is demonstrated using a well-defined artificial light-harvesting molecule featuring an iron(II) ion – a compound class that is known for their rapid population dynamics.

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HFSP reference number: LT0010/2024-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2024
Fellow: KITZMANN, Winald
Host supervisor: SCHLAU-COHEN, Gabriela

A PHENOPUSHING PLATFORM TO IDENTIFY COMPOUNDS THAT FAST-TRACK CELLULAR ADAPTATION TO HYPOXIA

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Abstract

Maintenance of cellular function requires adaptation to external stressors, such as hypoxia. To date, drug discovery for protection against hypoxia has centered around specific targets (e.g., HIFs and PHDs). However, the complexity of hypoxia response raises the possibility of unexplored targets. Based on the observation that cells can adapt to hypoxia over time, we hypothesized that compounds that phenopush cells under acute hypoxia towards a chronic hypoxia state could fast-track hypoxia adaptation. We DEVELOPED PhenoDART (Phenotypic Discovery of Adaptive Responses and Targets), a screening platform for discovery of compounds that mimic an adapted state under hypoxia. An annotated compound library screen with PhenoDART successfully identified phenopushing hits that conferred functional protection against hypoxic stress, and highlighted enriched targets with evidence of involvement in hypoxia response pathways. Moreover, we demonstrate that this shift confers functional protection against hypoxia-related stress in orthogonal cellular assays. Overall, PhenoDART provides a framework for drug discovery aimed at alleviating cellular stress by fast-tracking adaptation.

HFSP reference number: LT000908/2020-C
HFSP Award category: ALUMNI Cross-disciplinary Fellowship
HFSP Award year: 2020
Fellow: LI, Li
Host supervisor: ALTSCHULER, Steven

SEARCHING FOR HYPOXIA SENSORS AND EFFECTORS USING A PHENOTYPIC PROFILING PLATFORM

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Abstract

Hypoxia plays a central role in the pathogenesis of high-altitude diseases, myocardial infarctions and stroke. Aerobic organisms have evolved the ability to sense and respond to changing O2 levels. PHD-HIF-VHL is the most well-studied cellular O2 sensing and response pathway, with the oxygen-dependent enzyme PHDs functioning as hypoxia sensor and HIF downstream targets functioning as the effectors. There are more than 200 O2-dependent enzymes in human. How many additional enzymes act as hypoxia sensors and regulate downstream cellular responses? Likewise, several dozen HIF target genes are well-characterized, but there are hundreds of HIF target genes with unclear roles in hypoxia response.

To identify novel hypoxia sensors and effectors, we combined image-based high-dimensional phenotypic profiling with machine learning to develop a platform that can distinguish normoxic and hypoxic cellular states. This platform enabled us to screen for perturbations that have epistatic effect with hypoxia. Through quantitative analysis of a focused screen of chemical perturbations on oxygen-dependent enzymes and HIF targets, we nominated potential hypoxia sensor and effector candidates. We further characterized the downstream regulatory pathways these components participate. Our study contributes to our fundamental knowledge of hypoxia response and provides drug targets for hypoxia-related diseases.

HFSP reference number: LT000908/2020-C
HFSP Award category: ALUMNI Cross-disciplinary Fellowship
HFSP Award year: 2020
Fellow: LI, Li
Host supervisor: ALTSCHULER, Steven

UNDERSTANDING THE METABOLIC FUNCTION OF ENDOPLASMIC RETICULUM IN PHYSIOLOGY AND DISEASE

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Abstract

As the largest organelle in mammalian cells, endoplasmic reticulum (ER) is dedicated to calcium storage, lipid biosynthesis, and folding and assembly of proteins destined for secretory pathway. ER dysfunction is associated with rare inborn errors such as ER storage disease, but also common diseases including diabetes, cancer, neurodegeneration, renal diseases, and cardiovascular diseases. Despite their essential roles across tissues, little is known about how metabolite, lipid and protein composition of ER varies to support cell- and tissue-specific functions and in response to disease. This is in part due to lack of technologies enabling rapid isolation and the study of ER functions. Given that ER only occupies a small fraction (~10%) of total cell volume, whole-cell or tissue metabolite profiling approaches inadequately represent ER metabolism, highlighting the need for robust and rapid methods to determine ER metabolites and proteins from mammalian cells and tissues.

In preliminary work, I designed a system and established an ER-localized marker for the rapid ER immunopurification (ER-IP) of ER-specific metabolites, lipids and proteins from specific cell types and complex tissues. Leveraging this approach, we aim to innovate in vivo genetic tools to map the ER metabolome and proteome in mammalian tissues, employing a multidisciplinary approach blending protein biochemistry, genetics, and metabolomics to construct a protein and metabolite list for mammalian ER in a cell- and tissue-specific manner. This will provide the foundation for systematic approaches to understand ER function and how its metabolome and proteome respond to different disease states and physiological alterations. Additionally, we will discover functions of new ER genes using a combination of genetic and biochemical techniques. Initial fundings have already pointed out the importance of maintaining ER redox balance in regulating ER disulfide bond formation, protein folding as well as initiation of unfolded protein repones. Ultimately, our research will create the first comprehensive atlas of tissue and cell-type specific ER functions and discover new ER gene functions, paying the way for groundbreaking discoveries in organelle biology.

HFSP reference number: LT0039/2023-L
HFSP Award category: AWARDEE Long-Term Fellowship
HFSP Award year: 2023
Fellow: Liu, Shanshan
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TELOMERE LENGTH IN A SOUTH AFRICAN POPULATION CO-INFECTED WITH HIV AND HELMINTHS

Engelinah Macamo*[1]; Zilungile Mkhize-Kwitshana[2]; Zamathombeni Duma[2]; Julian Mthombeni[3]; Pragalathan Naidoo[2]

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Abstract

Introduction: Biological aging, also referred to physiological and functional aging, refers to the constant decrease in immune system resulting in cellular damage and apoptosis overtime. One of the biomarkers of biological aging is telomere length. Limited studies have associated shorter telomere length with HIV and parasite single infections, with no studies reporting whether individuals with HIV and helminth parasite co-infections are at an increased risk of telomere length shortening. Aim: To investigate whether telomere length shortening is accelerated in a South African population co-infected with HIV and helminths compared to participants singly infected with either HIV or helminths, and to compare telomere length data with participants biochemical and full blood count parameters. Methodology: A total of 200 participants were subdivided into uninfected control (n=50), HIV single infection (n=50), helminth single infection (n=50) and HIV and helminth coinfection groups (n=50). Relative telomere length (RTL) was determined using Real-Time PCR, and associated with biochemical and full blood count parameters using multivariate regression analyses models that were adjusted for confounders. Results: The uninfected control group had the highest mean RTL (1.31±0.75) followed by the HIV-infected (0.96±0.42) and coinfected (0.93±0.41) groups which had similar RTL, and lastly the helminth-infected group (0.83±0.33) which had the lowest RTL ($p<0.0001$). Regarding biochemical parameters, when compared to the control group (reference group), a significant association between RTL and blood iron (β=-0.48), ferritin(β=-0.48), transferrin saturation (β= -0.57), transferrin(β=-0.57), phosphate(β= -0.47),vitamin A(β=-0.49) and C-Reactive Protein(β=-0.52) were noted ($p<0.05$). Regarding full blood count parameters, when compared to the control group (reference group), a significant association between RTL and hamoglobin(β=-0.47), haematocrit(β=-0.46), Mean Corpuscular Volume(β=-0.47), lymphocytes(β=-0.45), Mean Corpuscular Haemoglobin Concentration(β=-0.45), Red Cell Distribution Width(β=-0.47), monocytes(β=-0.45), eosinophils(β=-0.45), basophils(β=-0.44) and transferrin saturation(β=-0.57) were noted ($p<0.05$). Further results that correlates dietary intake and telomere length will be discussed. Conclusion: Accelerated biological aging, as indicated by telomere length shortening, is associated with HIV and helminth coinfections.

HFSP reference number: MACAMO,Engelinah
HFSP Award category: Other
HFSP Award year: 2023
Principal Investigator: MACAMO, Engelinah (South Africa)
Co-Investigators: Mkhize-Kwitshana, Zilungile (South Africa), DUMA, Zamathombeni (South Africa), MTHOMBENI, Julian (South Africa), NAIDOO, Pragalathan (South Africa).

INTRA AND INTER-SPECIFIC VARIABILITY OF MECHANICAL PROPERTIES IN CEPHALOPOD BEAKS

Anastasiia Maliuk*[1]; Evans Susan[1]; Isabelle Rouget[2]; Anthony Herrel[3]; Mehran Moazen[1]; Souquet Louise[1]
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Abstract

Cephalopods, encompassing over 800 species, are a diverse group of marine predators distributed across all marine habitats worldwide. Among them, the predominant coleoid family, characterized by the absence of an external shell, includes well-known representatives such as octopuses, squids, and cuttlefish. Coleoids utilize a buccal mass located within their arm crown for feeding, comprising a radula common to all molluscs, a set of masticatory muscles, and a pair of chitinous beaks devoid of mineralization. The beaks are responsible for breaking down the food items into small pieces. As their esophagus passes through their donut-shaped brain, the mechanical function of their beaks is crucial for preventing neural damage during ingestion.

Cephalopods exhibit a varied diet, including fish, crustaceans, and bivalves, requiring robust beaks to bite into these relatively hard prey items. Previous studies have highlighted a stiffness gradient in the beaks, and exceptional stiffness in the rostrum of the Humboldt squid (*Dosidicus gigas*), reaching a elastic modulus of 9-10 GPa, making it one of the stiffest organic materials worldwide. In other species such as the common cuttlefish (*Sepia officinalis*) and octopuses (*Adelieledone polymorpha* and *Pareledone turqueti*), studies show different stiffness values (8 GPa, 4.5 GPa, and 5.5 GPa, respectively). If linking the rostrum stiffness to different diets can be tempting, these studies lacked intra-specific variation analysis. The question then is: Is diet-induced mechanical pressure responsible for the observed variation in rostrum stiffness, or is it an artifact of intra-specific variability?

To answer that question, we selected a set of commercially available species and extracted the beaks of adult specimens of various sizes. We measured the animal mantle, beak, and rostrum length. We embedded all beaks in resin, polished the samples to expose the sagittal sections, and nano-indented the rostrum area to calculate the elastic modulus. In our sample, the rostrum stiffness is heterogenous with no gradient in this area. An important intra-specific variation of more than 1 GPa is observed in all species, yet not correlated with size. A difference in mechanical properties can be observed between species. The potential implication of size and diet in these rostrum stiffness variations is discussed.

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HFSP reference number: LT000476/2021-L
HFSP Award category: Other
HFSP Award year: 2023
Fellow: SOUQUET, Louise
Host supervisor: MOAZEN, Mehran

SINGLE-CELL IMAGING OF EXTRACELLULAR ELECTRON TRANSFER CORRELATED WITH GENETIC, BIOCHEMICAL, STRUCTURAL AND COMPUTATIONAL RESULTS REVEALS THE IDENTITY OF NANOWIRES ON COMMON SOIL BACTERIA

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Abstract

Microbial extracellular electron transfer (EET) via "nanowires" is invoked to explain various globally important environmental processes and applications that require long-range (>10 μm) EET, such as biogeochemical cycling of carbon, nutrients, and metals as well as bioremediation of toxic organic and metal contaminants in the groundwater. Diverse microbes have been proposed to use Type IV pilus (T4P) as "nanowires" for EET to Fe(III) oxides and electrodes (1), in part because disruption of the pilin subunit, PilA-N, disrupts EET. However, electron-conducting bacteria also produce filaments comprised of cytochromes OmcS, and OmcZ, which have been proposed as nanowires (2). All prior studies have been limited to genetic or structural studies or determining the conductivity of filaments, and none have directly demonstrated which of these filaments, T4P or cytochrome filaments, are responsible for EET in living cells (2). The PI's group has determined atomic structures of both T4P and cytochrome filaments from *Geobacter*, revealing a mechanism for electron transfer in the cytochrome filaments via a chain of stacked hemes, but no obvious electron transfer pathway for the T4P. Though these results strongly support the cytochrome filaments as being the true nanowires, without physiological studies this idea remains controversial (1).

To address this knowledge gap we used electron-imaging (3), to directly visualize EET along nanowires of living bacteria to minerals and electrodes at a single-cell level. By correlating biochemical and genetic analyses to EET, we show that *G. sulfurreducens* use cytochrome nanowires and not T4P for EET. We show further that cells switch nanowires depending on the electron acceptors, producing OmcS nanowires during mineral growth and OmcZ nanowires during growth on electrodes. Importantly, alterations to the pili impact the ability of *G. sulfurreducens* to conduct electrons indirectly by disrupting cytochrome nanowire secretion rather than by directly disrupting pilus-mediated electron transfer. We provide evidence that T4P function as secretory machines to assemble cytochrome nanowires, rather than as the nanowires themselves. Our studies resolve a long-standing controversy about the identities and functions of microbial nanowires and highlight the need to evaluate the conductivity and EET capability of intact filaments by living bacteria.

Web: ***Nature*** News & Views, Tweetorial;EurekAlert! Live Science, Structure Tour, *Nature Nanotechnology*

HFSP reference number: RGP017/2023
HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2024
Principal Investigator: MALVANKAR, Nikhil (USA)

IT TAKES TWO TO TANGO—UNRAVELING TROPHIC INTERACTIONS IN THE PHYCOSPHERE

Alejandro Martínez-Calvo*[1]; Carolina Trenado-Yuste[1]; Xi Li[1]; Bruce Wang[1]; Anushree Malik[2]; Ned S. Wingreen[1]; Sujit S. Datta[1]

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Abstract

In nature, microbes frequently self-organize into spatially structured communities, where different types of cells inhabit distinct spatial domains. This spatial arrangement plays a pivotal role in influencing various biological functions, including community growth, stability, metabolite cross-feeding, and diversity. An important example is the *phycosphere*—the region around phytoplankton (e.g., cyanobacteria, microalgae) in the ocean, in which trophic interactions with surrounding bacteria strongly influence carbon/nutrient cycling and aquatic food Webs. Laboratory studies typically focus on these microbial systems in well-mixed cultures, which provide valuable information on cellular processes, but do not capture the spatial arrangement of different cell types often found in nature. Thus, here, we address this gap in knowledge using direct visualization of spatially-structured bacteria-cyanobacteria communities in transparent hydrogel matrices. Our experimental platform enables byproduct exchange, mirroring the interactions and spatial organization found in diverse marine and terrestrial ecosystems. Our experiments reveal the emergence of complex dynamical spatio-temporal interactions between bacteria and cyanobacteria, driven by the exchange of byproducts. These interactions can be either beneficial or antagonistic for the communities. These dynamics strongly depend on environmental conditions, cell motility, and cell density, which we recapitulate with a minimal theoretical model. Our results provide quantitative principles to predict and control the trophic interactions in the phycosphere that play crucial roles in the environment and global ecology.

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HFSP reference number: LT00035/2021-C
HFSP Award category: AWARDEE Cross-disciplinary Fellowship
HFSP Award year: 2021
Fellow: MARTÍNEZ-CALVO, ALEJANDRO
Host supervisor: DATTA, SUJIT S.

A HIGH-THROUGHPUT SCREEN FOR THE IDENTIFICATION OF NUCLEOLAR LOCALISATION SEQUENCES IN RIBOSOMAL PROTEINS

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Abstract

The ribosome is a macromolecular machine common to all forms of life. It reads the information encoded in messenger RNA (mRNA) to assemble amino acids into a specific protein. The ribosome itself is composed of RNA and proteins. In eukaryotes, 80 proteins and 4 ribosomal RNAs (rRNAs) must come together in a special compartment within the nucleus, the nucleolus, to form a complete ribosome. While ribosomal protein entry into the nucleus is relatively well understood, how ribosomal proteins are localised to the nucleolus remains to be elucidated.

Here we present a high-throughput screen to identify nucleolar localisation sequences (NoLSs) in ribosomal proteins. The sequences of all ribosomal proteins are screened for NoLSs using a sliding window approach, followed by fusion of the resulting peptides to a fluorescent protein. The intracellular localisation of all fusion constructs can then be identified by automated live cell imaging of generated stable cell lines. The approach is theoretically extendable to screening for any peptide sequence that determines intracellular protein localisation.

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HFSP Award category: ALUMNI Long-Term Fellowship
HFSP Award year: 2020
Fellow: MEYER, Katrina
Host supervisor: KRAUSHAR, Matthew

GENEALOGY VS CONVERGENCE IN EVOLUTION OF INTEGRATIVE SYSTEMS: HOW TO MAKE A CIRCUIT AND A BRAIN?

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Abstract

How to make a neuron, a synapse, and a neural circuit? Is there only one 'design' for a neural architecture with a universally shared genomic blueprint across species? The brief answer is "No." I will provide evidence for neural systems' convergence and parallel evolution using several interdisciplinary approaches, from sequencing aboard oceanic vessels (Ship-Seq) and single-cell multiomics to behavior. Four early divergent lineages independently evolved distinct neuroid-type integrative systems from a nerveless common ancestor. Synapses also evolved more than once. Neuronal centralization and formation of the complex brain occurred at least 20 times independently. The first neural systems were peptidergic, with predominant volume transmission using at least several dozen signaling peptides. Multiple origins of neurons from secretory cells explain the observed molecular diversity of neural systems and non-synaptic integration of behaviors as the ancestral state. This scenario also explains the lack of homologs in peptidergic systems across the earliest branching animal lineages. Little-explored examples of convergent neuronal evolution in representatives of early branching metazoans provide conceptually novel microanatomical and physiological architectures of behavioral controls in animals with prospects of neuro-engineering and synthetic biology.

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HFSP reference number: RGP0060/2017
HFSP Award category: ALUMNI Research Grant
HFSP Award year: 2017
Principal Investigator: YOSHIDA, Masa-aki (Japan)
Co-Investigators: Edsinger, Eric (USA), MOROZ, Leonid L.(USA)

SPATIAL TRANSCRIPTOMICS DEFINES INJURY-SPECIFIC MICROENVIRONMENTS IN THE ADULT MOUSE KIDNEY AND NOVEL CELLULAR INTERACTIONS IN REGENERATION AND DISEASE

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Abstract

The identity and function of an individual cell is largely defined by its own gene expression. However, the local cellular composition and unique interactions within the surrounding microenvironment plays an important role shaping cellular behaviors. Therefore, incorporating information on the physical locations of cells within the tissue can facilitate the detection of functionally relevant cellular phenotypes. Here, we leveraged spatial transcriptomics to study the coordinated changes in cellular composition and gene expression profiles following Acute Kidney Injury (AKI). AKI represents an abrupt loss of excretory kidney function caused by multiple factors such as ischemia, sepsis, or nephrotoxic drugs. AKI disrupts the intricate renal architecture and triggers limited regeneration, inflammation and fibrosis. We applied sequential Fluorescence In Situ Hybridization (seqFISH) in a mouse model of AKI to detect changes in gene expression and cellular interactions within injured and control kidneys. We measured the expression of 1300 genes in >200,000 single cells in situ and identified 22 cell types encompassing all major kidney populations as well interstitial and immune cells, vasculature, and a specific population of injured epithelium. This data represents a comprehensive map of the cellular, molecular and structural changes following AKI. Using our spatial information, we discovered that fibroblasts show injury-specific and spatially-dependent gene expression patterns, which were not detected when we removed spatial information and considered gene expression alone. We identified *Clcf1-Crlf1* interactions between injured epithelial cells and fibroblasts are highly spatially localized and constitute a key determinant of injury. We further detected cellular microenvironments resembling Tertiary Lymphoid Structures (TLS), comprised of immune cells and fibroblasts with distinct gene expression. These TLS-like microenvironments contained mainly CD4 T cells and T-regs as well as macrophages with a pro-inflammatory phenotype, compared to their counterparts located outside these structures showing a more regulatory gene expression. Taken together our experimental methodology and analysis demonstrates that spatial microenvironment information enables discovery of distinct spatially dependent molecular and cellular mechanisms associated with kidney injury, repair and disease.

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HFSP Award year: 2019
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WIDESPREAD ELECTRICAL PLUGS THAT TURBOCHARGE NANOWIRES OF ELECTRIC BACTERIA TO POWER NATURE'S ELECTRIC GRID: DIVERSE SPECIES CAN USE A SINGLE PROTEIN FAMILY TO INJECT ELECTRONS INTO CYTOCHROME FILAMENT TO EXPORT ELECTRONS TO EXTRACELLULAR ACCEPTORS

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Abstract

Deep in the ocean or underground, where there is no oxygen, *Geobacter* "breathes" by projecting protein "nanowires" into the soil to dispose of excess electrons resulting from converting nutrients to energy. These nanowires endow bacteria with the potential to perform environmentally important functions such as cleaning up radioactive sites and generating electricity. Although it has been known since 2002 that *Geobacter* makes surface filaments to dispose of electrons, only recently did the Malvankar group and others reveal the atomic structures of these filaments, which are polymers of cytochromes OmcS and OmcZ that form a chain of metal-containing heme molecules to carry electrons (1).

Although these structures explain how electrons move through nanowires, how bacteria move electrons to the nanowire remains a mystery, as most cell surfaces are electronically non-conducting. It was thought that a family of membrane-embedded "porin cytochromes" feed intracellular electrons to surface-displayed nanowires (2). Yet bacteria can transmit electricity even in their absence.

With HFSP support, the Malvankar and Salguero labs showed that a souble cytochrome transfers electrons to OmcS while still in the periplasm (3). They measured the redox potential of purified OmcS nanowires and found it comparable to that of a five-protein family that remains inside the bacterial periplasm, called **p**eri**p**lasmic cytochromes, (**Ppc)**ABCDE. They showed using Nuclear Magnetic Resonance spectroscopy that PpcA-E binds to OmcS and injects electrons, eliminating the need for intermediate electron carriers and explaining how cells transmit electrons at a remarkably fast rate (1 million electrons per second) despite electrons in proteins being able to move at rates at least 10 times slower.

The discovery that just two proteins, OmcS nanowires and one of PpcA-E, are sufficient to wire the inside of the bacteria to the outside greatly simplifies the model of how these bacteria export electrons by overcoming the slow electron flow among individual proteins. Malvankar Group also discovered that this minimal wiring machinery is ubiquitous in diverse species!

The team is also working on how another nanowire of cytochrome OmcZ is charged and identifying the role of porin-cytochromes in these processes.

Web: ***Nature Communications***
EurekAlert!
Physics World.
Behind the Paper

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HFSP Award category: AWARDEE Research Grant – Program
HFSP Award year: 2024
Principal Investigator: MALVANKAR, Nikhil (USA)
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MECHANOCHEMICAL CONTROL OF AVIAN GASTRULATION: THEORY AND EXPERIMENTS

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Abstract

During gastrulation, coordinated cell behaviors sculpt the vertebrate body plan. We previously quantified the tissue flows emerging from these multicellular dynamics in terms of dynamic attractors and repellers (M. S. et al. PNAS, 2020). We also linked cell behaviors to self-organized tissue flows using a theoretical model whereby gastrulation results from a mechanosensitive myosin instability (M. S. et al. Sci. Adv., 2023). Experiments and modeling determined that the attractor's shape depends on initial myosin patterns and cell ingression (M. C. et al. Sci. Adv., 2023). In recent work, we have also elucidated the mechanistic origins of morphogenetic repellers. We found that one repeller arises from the tug-of-war between the embryo and the extraembryonic tissue, while the second repeller self-organizes solely from the convergent extension of the mesoderm. By applying mechanical and chemical interventions inspired by our model, we were able to eliminate both repellers independently in chick embryos. Overall, our integrated modeling and perturbation approach reveals how coordinated cell behaviors sculpt a biomechanical landscape of attractors/repellers guiding avian gastrulation, elucidating the role of extraembryonic epiboly forces, embryonic apical constriction, ingression, and mechanosensitive myosin activity.

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HFSP Award category: AWARDEE Research Grant – Early Career (formerly Young Investigator Grant)
HFSP Award year: 2024
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GENOMIC CHARACTERISATION OF ACINETOBACTER BAUMANNII ASSOCIATED WITH NEONATAL SEPSIS IN A SOUTH AFRICAN POPULATION

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Abstract

BACKGROUND: Multi-drug resistant (MDR) hospital-acquired *Acinetobacter baumannii* is responsible for approximately 1.27 million deaths globally, with the highest disease burden in sub-Saharan Africa. Nevertheless, a paucity of genotypic characterization of *A. baumannii* is associated with childhood death. The study thus aimed to sequence the whole genome of invasive *A. baumannii* isolates associated with under-5 deaths in a South African population.

METHODS: Seventy-six *A. baumannii* strains were sequenced from post-mortem blood, lung tissue, or cerebrospinal fluid (CSF) samples collected from February 2018 to December 2022. Sequencing was limited to isolates from decedents where the infection was attributed by an expert panel to have contributed to the causal pathway to death. All isolates were cultured on CHROMagar plates, followed by genomic DNA extraction on BioMérieux NucliSens® easyMAG®, and sequencing performed on the Illumina Miseq Platform. The analysis involved comprehensive bioinformatics approaches for quality control, read assembly, Sequence Type (ST) determination, antibiotic resistance profiling, identification of insertion sequences, and virulence genotyping.

RESULTS: ST2 (47%) was identified as the predominant ST, followed by ST1 (39%), ST243 (8%), ST79 (1%), and ST25 (1%). All isolates were MDR, with specific genes conferring resistance to *β*-lactams (*bla*OXA-23(91%)) and aminoglycosides (ant(3")-lIa (100%), aph(6)-Id (89%), and aph(3")-Ib (89%)). Phylogenetic analysis revealed diverse genomes and the presence of multiple virulence factors (ompA, adeFGH, PANG, LPS, BfmRS, and PbpG) contributing to *A. baumannii* pathogenesis.

CONCLUSION: This study characterizes *A. baumannii* isolates associated with fatal infections in children under five. These findings underscore genomic surveillance's significance in mitigating morbidity and mortality in young children and implementing effect control measures against *A. baumannii*.

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HFSP Award category: ALUMNI Young Investigator Grant
HFSP Award year: 2024
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A QUANTITATIVE IN VITRO APPROACH TO VIRUS INFECTION

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Abstract

The study of phage biology and infection mechanisms is an important field of research that, since the first half of the 20th century, has delivered major scientific outputs for both fundamental scientific fields notably molecular biology and applied domains through applications like phage therapy.
Viral entry process that goes from phage fixation to viral DNA ejection inside the host plays a major role in the phage biology governing central aspects such as its infectivity and its specificity.
The central goal of my project is to achieve a functional reconstitution of this biological process in a controlled "in vitro" setup to provide a new way to gather quantitative knowledge on this process and its underlying mechanisms. The infection of highly controlled synthetic cells by viral particles would be a game changer in the study of phage infection mechanisms.
To achieve that goal, I am applying state of the art methods enabling the high throughput engineering of phage based on the use of the efficient cell-free transcription translation system (TX-TL). I am combining these approaches with biophysics methods enabling the construction of custom TX-TL mixture encapsulating synthetic cells with functionalized membranes able to interact with phage particles.
Achieving an infection of synthetic cells by phage particles will be a key step for the full reproduction of a phage lytic cycle in a fully controlled in vitro environment.

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HFSP Award year: 2024
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PALEO-EVO-DEVO: A PILGRIMAGE TO THE PAST THROUGH UNDERSTANDING THE PRESENT

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Abstract

As a discipline dedicated to exploring the evolutionary history of life forms, paleontology's development over the past 200 years has heavily relied on the discovery of fossils. Fossils serve as the sole remnants of ancient life forms and constitute the primary data source for modern paleontological studies, providing valuable morphological and/or (geo)chemical information. They offer direct insights into the anatomy of extinct organisms and aid in the reconstruction of their paleoenvironment and lifestyles. However, when it comes to understanding the mechanisms that drive evolution, paleontology faces limitations. This is why paleontology must collaborate with modern biology, or neontology, to comprehensively address evolutionary questions.

With the support of HFSP, I had the privilege to conduct research on the evolutionary development process of several important phenotypes characterizing the transition from non-avian theropod dinosaurs to birds by combining paleontology with developmental biology. By investigating the tooth loss process during the ontogenetic development of the non-avialan theropod dinosaur *Limusaurus inextricabilis*, we demonstrated the evolutionary origin of the keratinized beak seen in modern birds. Furthermore, through the discovery of bizarre branch structures in amber-embedded feathers and their comparison with modern feather development, we explored how various differentiations of medullary tissue, in addition to branching patterns, contributed to the previously undisclosed morphological diversity of feathers in the Mesozoic era. By studying the regression process of a series of characters during the early evolution of birds, we attempted to elucidate how the degeneration of existing morphological characters, traditionally overlooked subjects, contributes to increasing phenotypic diversity.

Therefore, the cross-disciplinary field of Paleo-Evo-Devo has evolved to incorporate aspects of comparative anatomy, cell and molecular biology, developmental biology, and comparative genomics within a phylogenetic context, making significant contributions to existing evolutionary theory. More disciplines will likely be inspired by these interdisciplinary studies.

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HFSP reference number: LT000728/2018-C
HFSP Award category: ALUMNI Cross-disciplinary Fellowship
HFSP Award year: 2018
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EARLY REPLACEMENT OF SOMATIC CELL LINKER HISTONES CAUSES GLOBAL CHROMATIN DECOMPACTION AT INITIATION STAGE OF IPSC REPROGRAMMING

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Abstract

Differentiated cells can be reprogrammed into the pluripotent state by over-expressing transcription factors OCT4, SOX2, KLF4 and c-MYC over the course of 21 days, although the efficiency is very low. To understand the chromatin changes during reprogramming, we combined live-cell imaging and single-molecule tracking to assess the dynamics of core histone H2B and SOX2 in single cells along reprogramming trajectories. Pluripotent cells harbor a hyperdynamic chromatin, whereas somatic cells contain more static heterochromatin domains resistant to reprogramming. We investigate how somatic cells acquire hyperdynamic chromatin during reprogramming to pluripotency and how changes in chromatin affect scanning and site targeting by transcription factors. Surprisingly, we identify an early, global chromatin decompaction independent of the reprogramming status, within four days of reprogramming from human fibroblasts. The de-compacted chromatin persists specifically in reprogramming cells from day 7 and beyond and correlates with increased cell division. Mechanistically, we found that this early global chromatin decompaction is caused by direct repression of somatic cell-specific linker histone *H1.0* by the reprogramming factors, and integration of cell-cycle dependent linker histone H1.4 and H1.5. The global changes in chromatin precede the increased residence time of SOX2 and more sampling of pluripotency targets, indicating that early acquisition of hyperdynamic features favorable for chromatin scanning by pioneer factors enables full reprogramming.

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