HFSP AWARDS 2019

RESEARCH GRANTS ABSTRACTS

Research Grants (Program Grants and Young Investigators) are listed separately, alphabetically. The first named for each award is the Principal Investigator.

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Title: The repeatability of the genetic mechanisms underlying behavioral evolution

Abstract: Keen observers of nature have often wondered why diverse species seem to behave similarly. For example, different species of Hawaiian spiders spin similar web architectures, diverse anoles lizard species bob their heads with the same styles and speeds, and distinct species of damselflies avoid predators using the same techniques. These and many other striking examples are thought to represent the convergent evolution of behaviors. Does this convergence reflect changes in the same genes or does evolution act through many genetic routes to create the same behaviors? Genetic differences clearly play a role in behavioral variation, but it remains challenging to identify the genes that underlie evolution of behaviors. However, convergence in the genetic basis of developmental or physiological traits has been discovered with many specific examples of genes and mechanisms, so it is possible to use studies of convergence to discover how behaviors evolve. Therefore, we will use a powerful comparative system to discover the genes and molecular mechanisms that underlie convergent evolution of behaviors for the first time across divergent animals.

The Caenorhabditis nematodes offer a unique experimental platform to connect behavioral differences to genetic differences. Starting with the keystone model organism, C. elegans, and existing data, we will characterize and classify genetic differences across wild isolates from three species of Caenorhabditis - C. briggsae, C. elegans, and C. tropicalis. Whole-genome genotype data combined with high-throughput, high-content imaging of behaviors from these same wild isolates will be input into unsupervised machine learning algorithms to create a high-resolution genotype-phenotype map for a range of natural behaviors and examples of convergence. This map will be queried for signatures of shared genetic changes at orthologous genes to identify which variants are most important evolutionarily. The result will provide the first systematic glimpse into the genomic “knobs” that control behaviors at single-variant resolution across species and insights into the repeatability of the evolution of behaviors.

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Title: Studying sea urchin dermal photoreception to unravel principles of decentralized spherical vision

Abstract: Sea urchins are marine animals genetically close to the vertebrate lineage. Being eye-less and lacking a central nervous system (NS), these animals instead feature dermal photoreceptors dispersed over their spherical body surface and feeding into a decentralized NS. However, sea urchins can visually resolve objects and move towards them, and they can detect looming visual stimuli from any direction and accurately point their spines towards them. Such performance is normally associated with proper eyes feeding information into a brain. Sea urchins thus offer access to a unique visual system of a type that to date has not been studied in terms of its information processing. This alternative solution to vision may also have potential biomimetic applications for robotic miniaturization, smart probes, and intelligent materials where dispersed light detectors control the properties of the material.

The core of the proposed project is to investigate and model the neural mechanisms of information processing, which enables sea urchins to perform spherical vision by deploying an obviously very different mechanism from today's technology, and also very different from visually guided behavior in most other animals. Our study includes molecular and morphological identification of cell types, measurements of behavioral responses and electrophysiological photoreceptor responses, mapping the connectome of sea urchin photoreceptors and NS, and theoretical modelling of the information processing underlying visually guided behavior. We will map the connectomics of the NS and record the activity from key positions in the processing of visual information and generation of locomotory responses. The data will be used for computational modelling of the entire process from visual input to motor control. Special focus will be given to behavioral decisions where small changes in stimuli cause behavioral switches. We will also use genetic approaches to test the agreement between theoretical models and actual behavior.

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Title: nFlare: an innovative light approach to study and modulate neuronal activity in vitro and in vivo

Abstract: To understand how the brain represents the world is a central theme in neuroscience. Neural circuits encode information in terms of rate, timing and synchrony of action potentials arising from the activity of a complex spatial organization of excitatory/inhibitory neurons. The identity of the active neurons at any given time has a profound effect onto the final outcome and regulates fundamental human psychophysical behavior. Attempts to decode the complex behaviors in neurons has led to development of several neuromodulation and sensing tools, by which neural activity can be controlled by microelectrodes or through genetically altering specific neural circuits, ultimately to deliver electrical impulses. Unfortunately, these techniques introduce strong perturbations to the observed system, without reaching the desired spatio-temporal resolution. Experimental approaches that allow non-invasive activation of specific phenotypic groups of neurons could introduce systematic variation of the timing of signals revealing how neural ensembles encode information. Here we propose a novel (nano)tool to alter membrane potential in specific neuronal phenotypes in a spatiotemporally controlled manner.

The nFlare project will develop a novel research paradigm in neuroscience based on a new class of injectable nanodevices delivered to neurons and anchored to their membranes. These nanotools have the ability to depolarize cell membranes or to deliver active biomolecules. Electric or chemical stimulation of single cells will be achieved using deep-penetration near infrared excitation light, to reduce invasiveness.
nFlare core aims are: i) the development of nanodevice components able to generate photoelectrochemical current upon illumination with light; ii) state-of-the-art biochemical functionalization of the nanodevice to target specific neurons and to reduce inflammatory response; iii) applicability of such nanoscale devices as high spatio-temporal neuronal activity modulators in 2D and 3D in vitro neuronal networks followed by in vivo validation.
Selective modulation of neural circuits by artificial stimulation of neuronal membrane or controlled delivery of environmental factors could help answering fundamental neurobiological questions.

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Title: An integrative approach to decipher flowering time dynamics under drought stress

Abstract: Plants live in an ever-changing environment which is not always compatible with their survival. A major life threatening condition is drought stress. While most plants can deploy an array of physiological countermeasures to endure remarkable levels of stress, there is a huge variability in the different strategies that plants choose to adopt to deal with drought stress. Many plants evade detrimental stress conditions by activating their reproductive development (flowering) earlier compared to non-stress conditions, a strategy known as drought escape (DE). Notably, even in the most water-rich environments plants face unpredictable dry periods, yet how this information affects the floral network at the molecular levels is unknown.

We will address this question with a blend of molecular and genetics-based approaches. We will leverage known mutants with altered DE to identify candidate mechanisms that are drought sensitive and can act as molecular switch to activate flowering. To comprehensively define DE mechanisms utilized under natural conditions we will assess the variability of the DE response in natural plant populations and in crops, which were selected by human intervention for the different field scenarios. Our targeted large-scale screens will allow us to identify naturally occurring variants in the DE process and decipher the molecular mechanism responsible for DE activation. This information will provide breakthroughs in our understanding of novel regulatory mechanisms that play a role in driving developmental adaptations across extremely variable environmental conditions, their natural genetic variation and the selective forces that maintain such variation in populations, an important aspect for predicting and dealing with the effects of climate change. Finally, because flowering time is a major component of yield potential in crops, the defined mechanisms will help us develop breeding strategies targeted for sub-optimal irrigation scenarios to produce crops with ameliorated performances under drought conditions.

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Title: Regrowing the brain: evolution and mechanisms of seasonal reversible size changes in a mammal

Abstract: Organisms need strategies to survive when conditions are hard. For mammals, winter is particularly difficult - they have to invest large amounts of energy into keeping warm, while food availability is low. For this reason, many mammals migrate or hibernate. However, what to do if you are too small to migrate long distance, burn your energy fast, and cannot hibernate? The common shrew is such a mammal and has evolved an astonishing strategy: each individual shrinks in winter by up to 20% and then regrows in the spring by about 13%. This size change, thought to allow shrews to survive on fewer resources because of the smaller size and linked lower energy requirements, include not just overall size, but specifically organs that do not usually change size in fully grown animals, such as the brain, heart and liver.

The process of neurological degeneration and regeneration is of great interest, since many central nervous system diseases (e.g., Alzheimer's, multiple sclerosis) involve degeneration, but ongoing research for therapies to reverse this process has been of limited success. As one of only a few recorded examples of mammalian brain regeneration, understanding how the shrew regrows its brain can accelerate research that leads to future therapies.
To answer the question of how the shrew shrinks and then regrows its brain, we will establish this unusual species as a new model, by studying the biological, molecular, biochemical and genetic processes behind this reversible size change. Besides establishing a database of information that can be mined and researched in years to come to discover the pathways that generate this cycle in the shrew, we will test a metabolic model of neurological change by artificially blocking molecular access to fats. Thus, the cross-disciplinary study of this wintering adaptation may help us understand more about regeneration in mammals in general, and the brain in particular.

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Title: Navigating the waters – A neural systems approach to spatial cognition in fish

Abstract: In 2014, the Nobel Prize in Physiology was awarded for the discovery of place and grid cells that process spatial cues in the mammalian hippocampal formation;, the key structure for both navigation and episodic memory1. Place and grid cells, and additional cell types form the central building blocks in the current circuit models of navigation in mammals. However, a cohesive picture of how these circuits compute space and enable navigation has not been achieved. In fact, emerging evidence suggest that these navigation circuits are highly diverse in cellular phenotypes and functionality; they do not only map aspects of space but also elements like sound, time, and reward. How neural systems of spatial cognition have evolved outside of the mammalian clade is not clear, and comparative studies are critically needed to gain insights to basic functional constraints and structural requirements underlying these neural circuits.

The international research team of four PIs proposes a broad comparative systems neurobiological approach using teleost fish for integrative studies on higher navigation circuits. These fish are ideal models because they have conquered diverse spatial ecologies and show highly specialized sensory adaptions. Also, their brains exhibit an overall lower complexity to mammals, and are highly accessible to experimental manipulation. To establish systematic research on teleostean spatial cognition, the project combines neuroethological, electrophysiological, neuroimaging, and computational methodologies. Introducing a powerful electrophysiological recording technology in freely-moving fish and generating long-needed anatomical atlas resources, the project analyzes four teleost species with differing spatial ecologies. The team will uncover how different sensory modalities like vision, perception of depth, and active electrolocation are integrated during spatial navigation tasks, thereby investigating how top-down mechanisms modulate sensory integration of spatial learning.
Finally, the team will test specific hypotheses developed in small-scaled laboratory setups in an unconstrained natural environment. Here, the group will measure the activity of neurons in freely moving fish that explore a coral reef habitat. This will be the first ever attempt to analyze brain activity underlying navigation in the wild. Altogether, the project will provide new perspectives on the evolution, function, and mechanism of memory systems in animal navigation.

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Title: In vitro reconstitution of synaptic plasticity: a minimalist approach

Abstract: Neuronal circuits store information through the mechanism of synaptic plasticity, a process where synaptic transmission is strengthened or weakened. Long-term potentiation (LTP) is a major form of synaptic plasticity. It requires both activation of CaMKII and subsequent trafficking of receptors and other proteins to the postsynaptic site. Despite extensive research, the causative relationship linking these two processes is still unknown. Here, Hayashi (live imaging and electrophysiology), Zhang (structure biology), and Lucic (cryoelectron tomography) will team up and take a unique minimalist approach to reconstitute synaptic plasticity from purified proteins. We will reconstitute postsynaptic density (reconstituted PSD or rPSD) on a glass substrate using a group of key scaffold proteins (PSD-95, SynGAP, SAPAP, Shank, and Homer) and receptor such as NR2B. Once a key process is found in minimal system, we will test if the same mechanisms work in intact neurons. Finally, we will investigate the persistent modification of the rPSD induced by the activation of CaMKII, which is expected to act as a hub for trafficking of various proteins. The network organization of the resulting complexes in vitro and in situ will be determined by cryo-electron tomography. The final goal of this proposal is to understand the minimum essential machinery for activity dependent delivery of postsynaptic proteins.

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Title: Phase separation of glycolytic machinery as a fundamental mechanism in energy metabolism

Abstract: Glycolysis is a fundamental energy metabolic pathway which consists of ten enzymatic steps. Unlike the mitochondrion, which is a membrane-bound organelle, glycolytic enzymes are soluble proteins in the cytosol. Based on biochemical evidence, glycolytic enzymes have long been hypothesized to form functional complexes to sustain the rates of glycolysis. This purported complex, called the glycolytic metabolon, was a subject of intense study and debate thirty years ago. Still today we do not yet understand how these purported complexes are organized in cells to sustain local energy metabolism, or their physiological importance. This gap in knowledge results from the fact that when glycolysis was being rigorously examined forty years ago, the techniques did not exist to conclusively answer these questions. The main challenge in addressing these questions lay then, as now, in the ability to both examine the localization of the glycolytic enzymes in living cells, while understanding the biophysical and biochemical mechanisms of their association, and its implications in cellular physiology. We have established a collaboration to address these fundamental questions by making use of our joint expertise in vitro reconstitution and C.elegans physiology, using the energy demands of the C.elegans synapse as a model system.

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Title: Synthetic biocompounds to direct neuronal circuit assembly

Abstract: The cerebral cortex is composed of distinct subtypes of neurons organized in circuits allowing high-order functions such as integration of sensory stimuli and sensorimotor transformations. These different neuronal subtypes are connected with neurons located both within and outside of the cortex. Intracortical connectivity is mostly mediated by layer (L) 2/3 neurons, which form synapses with other cortical neurons within and across areas; instead neurons located in L5B project to sub-cerebral targets and are responsible for cortical output.

While the molecular diversity of cortical neurons and their circuit organization is increasingly understood, it is still difficult to genetically manipulate cortical neurons based on which circuits they belong to; the ability to do so would, however, be a critical skill to repair circuits when they are affected by injuries or neurodegenerative diseases. To address this challenge, here we combine our expertise in developmental neurobiology (DJ) and in bioengineering (WL) to develop a strategy to manipulate gene expression in cortical neurons in a circuit-dependent manner. We do so by engineering artificial synaptic contact-dependent signaling cascades to drive new cellular features.

Specifically, we will:
1. Assess the in vitro molecular identity and connectivity of pure populations of L2/3 and L5B cortical neuronal types and manipulate these cellular features by direct reprogramming of L2/3 neurons into L5B neurons (Aim 1).
2. Manipulate gene expression and cellular features of L2/3 neurons in vitro in a synaptic-contact dependent manner by developing a synaptic version of the synthetic notch (synNotch) receptor system (synsynNotch) (Aim 2).
3. Manipulate axonal projections of specific populations of intracortically-projecting neurons in vivo using the synsynNotch system (Aim 3).
Together, these experiments will increase our understanding of the mechanisms controlling cell-type specific circuit assembly and allow us to functionally interrogate this process through circuit-specific manipulation of gene expression.

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Title: Single-molecule protein sequencing

Abstract: Protein sequencing remains a challenge for small samples. A sensitive sequencing technology will create the opportunity for single-cell proteomics and real-time screening for on-site medical diagnostics. We will use our expertise of single-molecule protein detection and material sciences to develop novel sequencing tools. In particular, we will use graphene mass sensors to measure the mass of proteins with sub-Dalton sensitivity. Utilizing this high sensitivity, we will measure the mass of protein fragments and identify the sequence of the fragments. We will also apply this method for detecting post-translational modifications of single proteins. Ultimately we aim to achieve sequencing of full-length proteins. This proof of concept will open the door to single-molecule protein sequencing and pave the road toward the development of a new, fast, and reliable diagnostic tool.

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Title: Tracking trade across symbiotic networks

Abstract: The world is characterized by an unequal distribution of resources. To cope, many organisms evolve symbiotic trade partnerships to exchange commodities they can provide at low cost, for resources more difficult to access. Such trade partnerships allow species to colonize extreme environments and survive resource fluctuations. While the ubiquity and importance of trade partnerships has been established, we do not understand the chemical, physical, and environmental stimuli mediating trade strategies, nor how organisms integrate this information to execute trade 'decisions'. This is largely because of the lack of tools to quantify symbiotic trade across space and time.

Combining biophysics, fluid mechanics, network theory and evolution, we will develop techniques to track, quantify and predict trade strategies in symbiotic networks formed between plants and their arbuscular mycorrhizal fungal partners – a globally ubiquitous trade partnership fundamental to all terrestrial ecosystems. By visually monitoring the trade of nutrients tagged with fluorescent quantum-dot nanoparticles across scales - from within individual fungal hyphae up to complex plant-fungal networks - we will ask: (1) how do oscillatory flow patterns within fungal networks act to regulate fungal trade decisions; (2) can the fungus manipulate its chemistry and physical architecture to maximize nutrient transport and trade benefits; (3) can trade strategies be predicted by environmental stimuli; (4) what is the influence of the external microbiome on trade behaviors.

Using high-resolution video to track fluorescently tagged nutrients within hyphae, we will be the first to test how the fungal symbiont regulates internal flows to mediate trade. We will develop 2D and 3D time-lapse imaging of network topologies to test the factors driving the optimization of fungal transport routes. We will use transformed in-vitro root systems with precisely controlled nutrient landscapes to correlate specific trade strategies with environmental conditions. We will push the frontiers of tracking trade in whole plant mesocosms by growing plant-fungal networks on transparent farming film, characterizing how synthetic microbiomes affect trade strategies. By integrating the state of the art in imaging, fluid mechanics, and ecological manipulations, we will achieve a quantitative and predictive understanding of organismal trade.

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Title: Do hydrocarbons induce membrane curvature in photosynthetic organisms?

Abstract: The cell membrane is a double layer of lipid molecules. It plays a critical role in protecting the cell from its environment and in separating the different processes that take place within its interior. Membranes must change their shape in order for the cell to function, especially during cell division, and this depends on membrane curvature. At present, cells are only known to induce curvature by accumulating lipids in one of the layers or using specialised proteins. Our goal is to investigate a new mechanism of inducing membrane curvature by accumulation of hydrocarbons in the middle of the lipid layers that has not been observed before in nature.

These hydrocarbons are like the components of diesel fuel, and are found in photosynthetic cyanobacteria and algae – some of the most abundant and widespread organisms on Earth. Production of hydrocarbons in cyanobacteria or other microbes could substitute for liquid fuels derived from petroleum. As well, cyanobacteria and algae release hydrocarbons into the environment, where they are degraded by other bacteria that clean up oil spills. However despite their environmental and biotechnological importance, the exact cellular role of hydrocarbons has not been determined.

We recently discovered that hydrocarbons are essential for maintaining optimal cell size, growth and division, processes that require cell membranes to curve and bend, and found that cells lacking hydrocarbons have lipid membranes that are less curved or flexible. We showed that hydrocarbons integrate into the cell membranes, and used computer simulations to predict that this induces membrane curvature. To investigate this further, we have assembled a team of scientists from the UK, Sweden and New Zealand. By combining our different skills, we will analyse how hydrocarbons affect the physical properties of algal and cyanobacterial membranes by 1) running computer simulations; 2) studying membranes purified from algae and cyanobacteria; and 3) carrying out experiments on live cells. Together, these simulations and experiments will allow us to explore and quantify how hydrocarbons affect curvature and other membrane properties, and so conclusively establish the role of hydrocarbons in cells. As well as improving our understanding of biology, this information will assist the use of microbes for biofuel production and oil spill cleanup.

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Title: Decoding the biomechanics of flight-tone based acoustic communication in mosquitoes

Abstract: The aerial courtship “dance” of mosquitoes has fascinated entomologists for over 150 years. This dance involves highly controlled variations in the frequency and intensity of flight-tones (i.e. sounds generated by the flapping wings) with concurrent changes in flight speed and direction, and enables recognition of conspecifics, display of fitness and transmission of mating interest. However, despite over a century and a half of research, significant knowledge gaps continue to exist in our understanding of this behavior. To decipher this courtship dance, entomologists have to integrate acoustic, energetic and flight information for untethered, free-flying mosquitoes, but the tools that can provide these data have, so far, not been available. In the current project, the two investigators combine their respective expertise in computational biomechanics and acoustics, and behavioral entomology, to generate unprecedented data and insights into the biomechanics and physics of courtship-associated acoustic communication in mosquitoes. In particular, by combining computational modeling with biological assays, the team will generate six-dimensional soundscapes of free-flying mosquitoes engaged in courtship and determine how these soundscapes are actively modified during courtship. We will also estimate for the first time, the energetic costs of courtship and mate-chasing, and the potential constraints this places on courtship behavior. Finally, the team will characterize the degree to which, carefully tailored exogenous sounds can alter and even disrupt courtship. The success of this novel approach could be transformative for future research into comparative auditory mechanisms of communication across a wide range of flying insects. In addition, the insights gleaned here could form the scientific foundation for novel insecticidal/surveillance traps and also lead to environmentally friendly strategies for diminishing mating success in mosquito species that are vectors for malaria, Zika fever and other devastating mosquito-borne diseases.

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Title: Unravelling an unusual biomineralization from nano to macro scale using advanced technologies

Abstract: Osteoderms are hard calcified tissues that form within the skin of some animals. They resemble bone, hence the name, but are fundamentally different in several respects. Crocodile and armadillo skin plates, and turtle shells are among the most familiar examples, reportedly forming a protective armour against external predators and aiding locomotion. However, although less visible, osteoderms are also present in many lizards.

In terms of their shape, spatial distribution, and interaction, lizard osteoderms show the highest diversity in the animal kingdom, yet we know little about what drives this extraordinary diversity, how it is controlled, or how it originated. It could be a biproduct of other genetic differences or, more likely, a natural optimization to enhance osteoderm function, protective or otherwise, under conditions specific to each lizard type.

This project brings together a multidisciplinary team of expert engineers, developmental and evolutionary biologists from the UK, Canada and France to investigate the mechanisms underlying the development, patterning, and evolution of osteoderms in lizards. The team will use a range of advanced techniques (e.g. genetic analysis, material testing, imaging, and computer simulations) to investigate lizard osteoderms from the first molecular signalling events and cellular interactions, through to organismal level. Osteoderm mechanical properties will be characterised both as single units and as sheets so as to understand their function during feeding and locomotion.

This is a basic science project focused on a novel biological tissue and its evolutionary implications, but with a systems approach that may shed light on pathological calcifications, as well as aiding the development of biomimetic materials and structures. Most importantly it will train the next generation of scientists, in a multidisciplinary and international setting, providing them with a fundamental knowledge of biological tissues and a diverse skillset with which to address the global challenges of 21st century.

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Title: Imaging viral RNA genome assembly with high spatial and temporal resolutions inside infected cells

Abstract: Sporadically, novel and potentially devastating pandemic influenza A viruses (IAVs) are generated through genome reassortment between human and animal co-infecting IAVs. Such pandemic viruses emerge as a consequence of the segmentation of IAVs genome into a bundle made of 8 distinct viral RNAs (vRNAs). However the molecular mechanisms of vRNA intracellular transport and assembly into vRNAs bundles, which are critical for reassortment, remain largely unknown. Our project aims to elucidate these fundamental aspects of IAV life cycle by developing innovative approaches.

We challenge the original model that newly synthesized vRNAs, in the form of viral ribonucleoproteins (vRNPs), are transported across the cytoplasm on Rab11-dependent recycling endosomes. Based on our recent work, we hypothesize that the concomitant transport and assembly of vRNPs is driven by their physical association with remodelled endoplasmic reticulum (ER) membranes and Rab11-dependent transport vesicles distinct from recycling endosomes.

We will set up a cellular system which resembles the natural respiratory tissue targeted by IAVs, while being amenable to simultaneous imaging of the endogenous Rab11 protein and tagged vRNAs. We will develop two cutting-edge and complementary imaging methods: dual-color single molecule fluorescence in situ hybridization (FISH) in live cells for the tracking of distinct vRNAs that diffuse concomitantly, and cryo-Focused Ion Beam combined with electron microscopy in situ hybridization (EMISH) to image individual vRNPs and their transport vesicles at molecular resolution. We will further assess the role of cellular ER-shaping proteins by performing CRISPR/Cas9-mediated knockdowns, and by monitoring changes in the viral-induced remodelling of ER and biogenesis of vRNP transport vesicles by live fluorescence imaging and cellular EM.

The proposed research will require the very close collaboration between three partners with distinct but complementary expertise. The approaches developed jointly are poised to revolutionize our understanding of IAV multi-RNA genome transport and bundling, and thus help in the broader goal of achieving better prevention and treatment of influenza disease. Additionally, the proposed technical developments in live cell FISH and cellular EM, will have impact on other fields of studies well beyond the scope of the proposed project.

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Title: Imaging sensory processing and memory storage in the octopus brain

Abstract: Octopuses have highly complex brains and are capable of many advanced behaviors that involve cognitive abilities. However, their brains and nervous system evolved completely independently from those of vertebrates, and it is largely unknown how the brains of such seemingly "alien" animals perform vertebrate-like sensory and cognitive functions with this distinct brain organization. In this proposal, we will study how visual sensory information is processed and stored in the octopus memory system. In order to overcome the technical obstacles to achieve this, we will bring together two labs with complementary expertise. The Niell lab studies the visual system of mouse, using calcium imaging of neural activity to understand how cortical circuits perform the computations that underly visual perception and behavior. The Hochner lab studies learning and memory in the octopus vertical lobe. They have used electrophysiological tools and behavior to show that the vertical lobe is organized in a simple fan-out fan-in architecture and demonstrates robust activity-dependent synaptic plasticity. However, these current experimental methods are not sufficient for understanding how learning and memory networks store sensory features that are likely represented sparsely in the activity of many individual neurons.

Together, we will implement two-photon calcium imaging techniques for the octopus brain, to directly observe how sensory information from the eye is processed and represented in the visual system as it is conveyed into the central brain. We will then measure how this information is stored in patterns of activity across the large population of small neurons in the memory centers of the octopus brain, within a learning paradigm. In other words, we will watch memories being formed from a visual input. We will also perform manipulations that will allow us to determine the role of synaptic mechanisms and neuromodulation that enable this storage and its modulation by reward and punishment signals. The result of this collaborative endeavor will be a comprehensive view of neural information processing, from sensory input to memory formation, in the unique and enigmatic brain of the octopus.

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Title: A spatiotemporal map of signalling processes controlling human stem cell renewal and differentiation

Abstract: The Personalized, Regenerative Medicine of the future will rely on being able to make replacement cells and tissues of choice at will and in a robust, predictive manner. However, key challenges have to be overcome before the promise of personalized stem cell therapeutics becomes a reality. This is because stem cell renewal/differentiation are stochastic processes, precluding the differentiation of a stem cell population into a homogeneously differentiated desired cell type, but also leading to spurious differentiation during renewal. This is thought to partly arise from heterogeneous single-cell signaling states among different cells of a population, which are not measurable using classical 'population-average' biochemical methods. A mechanistic understanding of how dynamic signaling processes control differentiation/renewal fates at the single-cell level might therefore significantly improve our capacity to robustly and precisely manipulate cell fates for tissue engineering purposes. We propose to use an integrated interdisciplinary strategy to map the dynamic, single-cell signaling programs that control differentiation/renewal using human Pluripotent Stem Cell (hPSC) differentiation into neural stem cells as a differentiation paradigm. Using multiplexed, genetically-encoded biosensors, we will quantitate hPSC single-cell dynamic signaling states by large-scale, multi-color, multi-day timelapse microscopy across millions of cells, to reveal with unparalleled precision how heterogeneous signaling states correlate with renewal/differentiation fates. Using computer vision approaches, we will automatically segment, track and lineage at scale each of the cells that were induced to self-renew or differentiate, and we will extract a panel of signaling, cell-cycle, pluripotency state, and cell morphodynamics features that quantify these dynamic processes. We will then mine these high-dimensional feature sets to build computational models that identify dynamic single-cell signaling patterns associated with robust fate transitions and predict actionable interventions that might cause those transitions. Lastly, using drug perturbations, and/or microfluidic/optogenetic actuators, we will quantitatively test those predictions by evoking synthetic dynamic signaling states that induce robust fate transitions. Our approach will help to significantly clarify the mechanistic basis of signaling-mediated human stem cell fate decisions, providing new avenues to robustly control stem cell fate. This might help establish a larger framework, broadly applicable to other hPSC lines and differentiation routes.

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Title: Elucidating the development of biological optical nanostructures

Abstract: Optical nanostructures are highly organized composites of materials with varying refractive indices (e.g. keratin, melanin and air) that produce some of the brightest colors found in nature through coherent light scattering. How these tissues organise themselves at the nanometer scale to produce colors is poorly understood, despite its fundamental significance to developmental and evolutionary biology and potential to spark advances in the biomimetic design and "green" commercial manufacture of self-assembling optical materials.

We thus propose to use both transcriptomic, laser diffraction and microscopy-based tools of developmental biology to elucidate the mechanisms by which these nanostructures self-assemble in a subsample of birds (Class Aves), a group with incredibly diverse structural colors and mechanisms. Our working hypothesis is that iridescent colors form through depletion-attraction, phase separation and other self-assembly mechanisms. Because most developmental biology is done at larger size scales, testing these hypotheses will require the use and development of methods such as wet cell TEM and in situ laser diffraction analysis to adequately resolve nanometer-scale changes in developing tissue. We will then test these proposed mechanisms using biomimetic approaches that replicate natural conditions as closely as possible (e.g. at room temperature, at biological pH) using natural or semi-natural materials. Use of optical techniques including angle-resolved spectrophotometry and microspectrophotometry will enable us to compare these properties between the natural and synthetic versions. This approach will enable us to not only experimentally test modes of development but also generate and test new materials and/or processes to produce them.

There are three highly innovative aspects to this proposal. First, it attempts to unlock the developmental pathways producing nanostructured tissues. This is a long-standing question with few answers thus far. Second, it uses biomimicry in novel ways to test developmental hypotheses and pushes the technical boundaries of developmental biology by focusing on nanometer-scale organisation of tissues. Finally, the use of biologically realistic chemistry in our biomimetic approaches is a huge leap forward in this field where most work is done at high temperature or with non-biocompatible materials. This work will therefore significantly advance both our fundamental understanding of these materials and the tools to study them and other nanoscale materials.

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Title: Enhancing mitochondrial DNA fidelity to improve mammalian lifespan and healthspan

Abstract: Animal mitochondrial DNA (mtDNA) has a higher substitution rate than nuclear DNA, with the accumulation of mtDNA mutations being one of the hallmarks of ageing. This discrepancy in the rates of evolution is partially due to the lack of mismatch repair activities in the mitochondria. Octocorals – a group of cnidarians – have a reduced rate of mitochondrial evolution and encode a MUTS-like protein (mt-MutS) in their mtDNA. Previous analyses suggested that this enzyme was acquired from a virus and has been universally retained among octocoral taxa. Its function, however, remains unknown. The project will combine comparative, structural, and experimental approaches to investigate the function of mt-MutS and to test whether mt-MutS expression results in lower mutation rates in mtDNA and improve heath in ageing. Comparative analysis of octocoral mtDNA will be used to identify distinct mutation patterns among its lineages and correlate them with the changes in mt-MutS. Partial mt-MutS sequences will be used to identify clades with unusual or accelerated rates of mtDNA evolution for additional sampling. Site- and taxa-specific evolutionary rates in mt-MutS will be analyzed to infer functional and structural constraints and to optimize the choice of the mt-MutS for transgenesis. Ancestral sequences of mt-MutS for the nodes of interest will also be reconstructed and analyzed. A structure-function approach will be utilized for in vitro dissection of mt-MutS functions. The full-length proteins from several species and a reconstructed ancestral sequence will be tested for stability and for amenable structure determination. Isolated domains will also be used for high-resolution structural analysis by diverse biophysical techniques to probe molecular details of binding and nuclease activity for understanding and improving function. Finally, we will generate transgenic mice that express a mitochondrially-targeted version of this optimized mt-MutS enzyme to test its effects on mtDNA mutation rate. The transgene construct will be knocked-in to mice by directed Easi-CRISPR template repair or BAC-transgenesis. mtDNA mutation rate analyses in wildtype and mice with enhanced mitochondrial mutation rates will be undertaken. An ageing study on mice expressing mt-MutS will determine if enhanced mtDNA fidelity can positively affect organismal lifespan and healthspan.

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Title: Communication and the coordination of collective behavior across spatial scales in animal societies

Abstract: We propose to use new tracking technology and computational modeling to determine how vocal communication influences collective behavior in animal societies. Canonical examples of collective movement such as bird flocking and fish schooling involve cohesive groups making short-term decisions in a shared context. However, many animals form stable social groups that coordinate and cooperate over extended time spans, across varying distances, and in diverse contexts. In these stable animal societies, group members must make decisions despite varying access to information and exposure to the costs and benefits of coordinating. Moreover their decisions are likely to be shaped by the long-term social relationships among group members. To achieve coordination in such systems, many species use sophisticated signaling systems, such as vocal communication, that transfer information among group-mates. Animals can flexibly control the vocalizations they produce independent of their movements, resulting in a complex interplay between signaling and movement that ultimately drives group-level outcomes such as collective decisions and coordinated actions.

To understand the mechanisms underlying coordination in animal societies, we will record movements and vocal signals concurrently from all members of wild animal groups at a high resolution, and across varying degrees of spatial dispersion. We will compare three mammal species that face a common set of coordination task, but differ in cohesiveness: meerkats form highly cohesive groups, coatis are moderately cohesive, and spotted hyenas live in fission-fusion societies. In each species, we will 1) fit at least one entire social group in the wild with tags that continuously record fine-scale movements and vocalizations, 2) combine supervised and unsupervised machine learning to identify animal calls and movement states, 3) develop modeling approaches to reveal how animals integrate spatial and acoustic information, how information flows through groups, and how social interactions give rise to collective outcomes, and 4) conduct audio playback experiments to isolate causal factors driving collective dynamics. Combining these approaches with long-term data from field studies will shed light on both unifying features underlying coordination mechanisms across animal societies and differences imposed by distinct constraints.

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Title: How complex behavior is encoded in the genome and wired in the brain

Abstract: Despite effort, it remains incredibly difficult to identify the cellular basis, and/or the causative genetic variants, underlying complex behavior. Understanding how behavior is encoded requires solving a dual problem involving both neurodevelopment and circuit function. Genes build nervous systems; nervous systems are activated to produce behavior. Streelman and Baier will collaborate to develop a unique model system to chart the complex path from genome to brain to behavior, in vertebrates from natural populations. In Lake Malawi, male cichlid fishes construct sand 'bowers' to attract females for mating. Bower building is an innate, repeatable natural behavior that we quantify in the lab. Males build two bower types: 1) pits, which are depressions in the sand, and 2) castles, which resemble miniature volcanoes. Species that build these two bower types can interbreed in the lab. Remarkably, first-generation hybrids of pit- and castle- species perform both behaviors in sequence, constructing first a pit and then a castle bower, indicating that a single brain containing two genomes can produce each behavior in succession. Moreover, brain gene expression in these hybrids is biased towards pit- alleles during pit digging, and castle- alleles during castle building. This phenomenon of allele-specific expression matched to behavior is compelling and offers the chance to identify the genome regulatory logic and neural circuitry underlying complex behavior. Streelman's group will use single-cell RNA-sequencing to pinpoint specific cell populations that mediate context-dependent allele-specific expression in male bower builders. Baier's team will use genome editing and optogenetic tools to manipulate the neurons that matter in the brains of behaving Malawi bower builders. Our collaborative work will thus identify the neurons responsible for biased allelic gene expression matched to behavior, and then manipulate those neurons to modify behavioral output. Achieving our goals will demonstrate how the genome is activated in particular cell types to produce context-dependent natural social behaviors.

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Title: Regulation of membrane receptor function in the brain by lipid composition and dietary inputs

Abstract: Approximately 30% of mammalian genes code for transmembrane proteins, which comprise the majority of signal receptors and transducers. These functions are not solely encoded in protein structure, but are also regulated by the unique physicochemical environment of mammalian membranes. A key unmet challenge is to understand the interplay between the composition of membranes, their collective physical properties, and their resulting effect on protein function. The knowledge gap is especially apparent for mammalian neural tissue, whose membranes are highly enriched in ω-3 polyunsaturated fatty acids (PUFAs), which our bodies do not synthesize. This composition is central to neural function as evidenced by brain lipid alterations in numerous developmental, psychological, and neurodegenerative disorders; however the mechanistic relationships between the brain's unique lipid composition and neurological functions are unknown. Major open questions are how neuronal function is influenced by the lipid content of the membranes that host neural signal transduction receptors, and how factors like diet and environment can influence those lipid compositions. Here, we assess the paradigm-shifting hypothesis that alterations of neuronal membrane lipid composition affect the signaling in the brain and contribute to the pathogenesis of neurological disorders. Particular emphasis is placed on the role of dietary lipids in modulating membrane composition, and the functional consequences thereof. Breakthroughs in understanding the central role of lipids will emerge from the project's interdisciplinary crosstalk between detailed comprehensive lipidomics, molecular computer simulations, quantitative cellular biophysics, and molecular neurobiology. We focus on two parallel research streams: pattern-recognition receptors and G protein-coupled receptors, which are here used as representative systems to explore the regulation of neural receptors by lipids in a pipeline involving computational, synthetic, and natural model systems, as well as cultured cells and in vivo studies. As the influence of lipids on neuronal receptor function has so far been almost completely ignored, these studies will generate significant impact. Further, the modulation of membrane composition by diet may provide important translational insights and drug-free therapeutic strategies.

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Title: Molecular approaches to study individually identified mechanosensory neurons of the leech

Abstract: To study how nervous systems arise and function, scientists use animal models in which it is possible to integrate research on fundamental processes across different levels of organization from genes to behavior, and from the zygote to the adult. The medicinal leech (genus Hirudo) provides one useful model, because its nervous system is much simpler and easier to work with than vertebrate or mammalian nervous sytems, even though it functions in a similar manner--Hirudo has been used to study phenomena of general importance such as: how glial cells function; how neurotransmitter are released; how synapses form and regenerate; and how neural circuits function to control behavior. Another leech (genus Helobdella) is used to study development and how development changes during evolution, giving rise to kinds of animals over hundreds of millions of years. These two leech species exhibit marked similarities of course, but also some differences. Technical considerations (small embryos for Hirudo; small adults for Helobdella) have made it difficult to integrate these two models, e.g. by applying molecular approaches in Hirudo or to study the adult nervous system in Helobdella. The goals of our project are: 1) to enhance the power of the Hirudo model by introducing newly-developed molecular approaches; 2) to implement approaches in Helobdella that will enable us to unite molecular and cellular approaches to developmental and behavioral neurobiology; 3) to develop new optical techniques for stimulating and recording neuronal activity without exogenous dyes or genetic manipulations.

The intellectual significance of the proposed work is twofold. First, it will enhance our abilities to answer fundamental questions regarding how nervous systems function and development by introducing cutting edge technical approaches to the cellularly simple, physiologically accessible leech models. Of equal importance, it will provide a new evolutionary perspective into neurobiology, by allowing us to examine similarities and differences between leech and other models, including arthropods, nematodes, and vertebrates which have all been evolving separately for more than half a billion years.

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Title: 3D atomic-scale movies of molecular machines in action

Abstract: Capturing the dynamic 3D atomic-scale structure of a macromolecular machine while it performs its biological function remains an outstanding goal of biology. Conventional structural tools (e.g. X-ray crystallography, NMR & cryoEM) only provide 'snapshots' of stable states along a reaction pathway. Reaction intermediates, and in particular short-lived intermediates, are hard to capture and characterize with such conventional techniques. Here we propose to combine (prior) information from multiple existing static structures of stable states with dynamic datasets of inter-atomic distances obtained by high-throughput non-equilibrium single-molecule FRET (smFRET) measurements in a microfluidic mixer using novel time-resolved multi-pixel single-photon avalanche diode detector. These measurements will be performed on libraries of molecular constructs, sampling multiple inter-atomic distances as function of reaction time. These measured distance distributions will then serve as multiple intra- and inter-domain distance constraints which, together with prior information (available structures), will enable the Rosetta software to achieve large-scale energy optimization-based refinement of time-resolved 'snap shots' of complex structures with improved accuracy. These time-resolved Rosetta structures together with intermediary molecular dynamics simulations will allow solving the 3D atomic-level structure of the macromolecule for each sampled reaction time point, eventually producing a 3D structural dynamic movie of the macromolecule in action. To demonstrate the utility of the proposed method, we will solve the dynamic structure of RNA polymerase during transcription initiation (promoter binding, bubble opening, abortive initiation, promoter clearance) and a pair of intrinsically disordered proteins (IDPs) involved in transcription regulation (ACTR and NCBD) that adopt a fully folded structure during a coupled folding and binding reaction. In addition to elucidating outstanding questions in transcription by combining detector developments, high-throughput and time-resolved out-of-equilibrium single-molecule FRET measurements with new experimentally-constrained molecular structure computational approaches, this multidisciplinary project will result in a new generic toolkit applicable to a large array of enzymes, proteins and molecular machines.

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Title: Spatiotemporal neurochemical dynamics of behavioral flexibility in the striatum

Abstract: The overarching goal of this proposal is to investigate the spatiotemporal coding of acetylcholine (ACh) and dopamine (DA) with high-resolution and precision in the striatum using state-of-the-art genetically encoded biosensors combined with modern optics in awake animal imaging. The striatum is crucial for movement, learning and flexible behavior, with striatal DA and ACh both playing key roles in these functions. While the role of DA is relatively well established, the role of ACh in natural behavior still remains enigmatic. Cholinergic interneurons (CINs), the major source of striatal ACh, are involved in processing contextual information that guides flexible behavior. Locally, CINs also exert control over striatal DA release, hijacking DA axons and making them release ACh by activating nicotinic receptors near their terminals. We propose to image the spatiotemporal dynamics of striatal DA and ACh using two-photon microscopy and endoscopy in awake mice engaged in tasks requiring behavioral flexibility. To image DA and ACh simultaneously during behavior we will extend the color-spectrum of DA and ACh biosensors. We will also further optimize the performance of these biosensors to make them suitable for robust in vivo application. Our combined interdisciplinary but complementary expertise – in biosensor engineering, imaging, modelling and behavior – is essential for our aims. We will ensure a coherent, interactive approach by sharing procedures, behavioral tasks, and biosensor technology, with regular planning sessions and feedback of results. A successful outcome of this program will reveal, for the first time, the spatiotemporal coding of neuromodulatory signaling by DA and ACh and how it shapes the function of striatal circuits during flexible behavior. We will also obtain a mathematical understanding of the genesis of the spatiotemporal dynamics. The newly engineered sensors developed in the program will have further broad applications in various biological systems of interest, which will ultimately pave the way toward a more complete understanding of brain function at synaptic, microcircuit, and behavioral levels.

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Title: Conversations between brain and vasculature: studying and mimicking their intertwined development

Abstract: The brain comprises billions of neural cells interwoven in a highly precise way and it is ultimately responsible for how we perceive, react to, and remember the world around us. We are fascinated by how the intricate structure of the brain is assembled step-by-step at an early stage of life ("embryonic development"), starting as a relatively shapeless mass within the nascent embryo and culminating in a highly complex organ in an adult. Specifically, we hypothesize that blood vessels that permeate the early brain have important roles in fostering brain assembly, and we further predict that they produce important cues that actively shape a specific early step of brain development. This is of great significance, because blood vessels were once thought only to passively supply oxygen and nutrients to various organs. We instead suggest an active role in which blood vessels function as a 'signaling center' to actively control the arrangement of neural cells during brain assembly.

We have assembled an intercontinental team to tackle this question via a three-part, integrated effort. First, we will create a map of where and when arteries and veins (two major types of blood vessels) first enter the nascent brain. Second, we will intermingle neural and blood vessel cells in a Petri dish and study their interactions. Third, we will block specific functions exerted by blood vessel cells and propose that this will have a commensurate impact on the spatial arrangement of nearby neural cells. These activities are innately highly collaborative and will involve interactions between the partner laboratories in each nation.

Taken together, we propose to explore how the brain is assembled during embryonic development and we will test whether blood vessels act not as passive support for, but rather as active executors of, this intricate process at early steps. If true, this has important ramifications. Not only will it shed light on how the early brain is assembled, but it adds to the emerging idea that neural cells and blood vessels share an intimate functional and spatial relationship, starting from the embryo and lasting into the adult — an idea pertinent to early brain assembly; our efforts to engineer this process in a Petri dish for regenerative medicine; and finally, the origin of certain neurological disorders that are caused in part by blood vessel dysfunction.

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Title: From DNA to K-fibers: probing centromere function in the genesis of age-related oocyte aneuploidy

Abstract: Cell division is a complex but fundamental life process. Among its many purposes, it is needed for a fertilized egg to develop into a human being, for our wounds to heal, for infections to clear and for our bodies to sustain life. Whenever a cell divides, its genetic information is duplicated and packaged into chromosomes which are then separated and equally distributed between the new daughter cells. For the newly formed cells and ultimately the body to be healthy, distribution of the chromosomes should be highly accurate. Accurate chromosome separation during cell division is driven by dynamic cellular cables that are connected to special chromosomal regions known as centromeres. Indeed, defects in centromere formation or function compromise chromosome separation and lead to daughter cells containing too many or too few chromosomes, a hallmark of cancerous cells.

When eggs are prepared for fertilization, a specialized form of cell division called meiosis separates the chromosomes. The accuracy of chromosome separation during meiosis determines whether a fertilized egg can develop into a healthy human being. Surprisingly, meiosis in humans and other mammals is highly prone to errors and often leads to eggs that contain the wrong number of chromosomes. Fertilization of such chromosomally abnormal eggs frequently leads to human embryo deaths and conditions such as Down's syndrome. Complications arising from erroneous chromosome separation in eggs become even more frequent as women get older. Research in the field of meiosis has only scratched the surface of why chromosome separation in eggs is highly error-prone. Furthermore, the reasons behind the deterioration in the quality of this process as women get older largely remain unknown.

In this research proposal, we will test the hypothesis that defects in centromere function that accompany ageing may contribute to poor quality of eggs in older women. To achieve this, we will combine our unique but synergistic expertise in advanced light microscopy, genome editing and electron microscopy. Knowledge gained from this study will advance our understanding of why eggs of older women are often chromosomally abnormal. In the long-term, this work can potentially be exploited for treatments of human infertility.

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Title: From synapses to networks. Understanding mechanisms of fear generalization across brain scales

Abstract: A shared feature of many anxiety disorders is the overgeneralization of fear, the tendency to falsely link together events that have differing emotional significance. In the case of abnormal fear learning this can produce heightened levels of arousal and fear in situations that should be perceived as safe.
Previous studies indicate several distributed brain areas interacting with neuronal networks in the amygdala to modulate the fearful perception of diverse stimuli. It therefore remains largely unknown how changes in synaptic and cellular function (synaptic scale) can influence encoding of fearful stimuli in large cell networks (network scale) and how interaction between brain areas (global scale) finally leads to overgeneralized behavioral responses. Although a few studies have begun to examine neural substrates of fear generalization at the synaptic and network level, to date, no structured and integrated picture has been generated. This major gap exists because no study so far has been able to integrate experimental results across the different scales to generate a complete model of interactions that regulate fear generalization. As a consequence, new therapies and drugs to treat anxiety disorders emerge only slowly with no major discoveries in sight.

In this proposal, we aim to utilize a unique combination of expertise in neurophysiology, optogenetics and advanced brain imaging to study the mechanisms of fear generalization across spatial scales, ranging from synapses to individual neurons, to large-scale, distributed networks across the whole brain. For the first time we will investigate how local amygdalar networks regulate fear generalization within the context of their afferent signals emerging from global brain activity. To connect synaptic, network and global scales, we will develop new innovative technologies such as an fMRI compatible version of a miniaturized microscope for functional Ca2+ imaging to simultaneously monitor whole brain function and local network activity during fear generalization tests in mice. We will complement these studies with in vivo Ca2+ imaging, optogenetics, and ex vivo electrophysiology to identify the distributed inputs and local circuits in the amygdala that generate overgeneralized fear responses.
The goal of our interdisciplinary systems neuroscience/engineering approach is to provide a comprehensive understanding of the neural substrates that trigger overgeneralized fear, thereby generating a novel framework for how anxiety-related behaviors emerge across different brain scales.

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Title: Exploration of the structure/function space of prebiotic to biological proteins

Abstract: Proteins have evolved to adopt many structures and perform diverse functions by exploring a sequence space spanned by twenty canonical amino acids (AAs). Whilst ten of the AAs were 'obvious' choices, as they abounded in the prebiotic world, the other ten were far less accessible prebiotically, thus provoking the question: Why (and how) were these AAs included in the genetic code, and was their inclusion prerequisite for protein evolution to be as successful as it has been? These seeming Gedankenexperiments are directly testable using an interdisciplinary approach we have devised.
Specifically, we propose to synthesize random protein libraries built from reduced (evolutionarily early) and alternative AA alphabets to compare the structure/function-forming potential of the proteinogenic and non-canonical yet prebiotically abundant AAs. The team of Stephen Fried will customize both commercial and home-made cell-free protein translation systems to express protein libraries composed of alternative AA alphabets. The team of Klara Hlouchova will use biophysical approaches to explore the structure-forming potential of the purified libraries. The capacity of the libraries to evince prebiotically-relevant functions will be assessed by the group of Kosuke Fujishima through selections that can relate genotype to phenotype (e.g., mRNA-display). Large sequence space (>10^12) will be analyzed in each experiment and because the same template libraries will be used, the outcomes of both the structural and functional studies will be directly comparable. This would not be possible without coordinated collaboration among the three teams.

Each team member enters the project with a key set of skills and scientific expertise. Klara and her team have a strong background in protein biochemistry, bioinformatics and experience with expression of protein libraries. Kosuke is an astrobiologist with strong experience in RNA molecular biology and his team takes a synthetic biology approach to studying peptide-RNA interactions. Stephen has experience in biophysics and synthetic biology and his newly started lab performs research in protein folding and engineering. This project relies on synergy of the above mentioned disciplines connected by our mutual interest in the origins of life, making it possible to address a broad fundamental biological question in a systematic way.

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Title: Evolutionary changes in human hosts and their pathogens during first contact in the New World

Abstract: In this project we will uncover the evolutionary dynamics in both humans and pathogens in response to epidemics. Recent technology advances enable detection of infectious agents in ancient DNA (aDNA) samples that tie to major historical epidemics. However, we know practically nothing about the dynamic process by which genetic adaptation occurs simultaneously in both the host and pathogen as a consequence of epidemic outbreaks.

We propose to integrate aDNA and sophisticated computational approaches to investigate the selective pressures imposed by the introduction of new pathogens during European colonization of the Americas. Our goals are to characterize the changes in both pathogen and human genetic diversity before and after European colonization to describe: 1) the genetic signatures that were putatively responsible for decimating the Native population, and 2) the selective and demographic processes that conferred adaptation to the colonization environment, especially with respect to pathogen exposure. To this end we will sequence the genomes of at least 30 individuals from before and immediately after colonization, and for the Colonial period we have access to archaeological remains of individuals who were likely victims of epidemics. We will also leverage the metagenomic data produced when sequencing aDNA and quantify the pathogen genetic diversity present in these samples before and after colonization. Lastly, we will use novel statistical methods to identify loci in post-colonization samples that depart from expected proportions of Native American, European or African ancestry to test whether admixture facilitated adaptation.

Our study will leverage temporal genomic data to address a long-standing question of how pathogens have influenced human evolution. As we lack studies quantifying jointly the changes in both pathogen and human diversity across time, this project offers a unique opportunity to directly assess, for the first time, how much evolutionary pressure is experienced within a human population when encountering new pathogens. Our design integrates novel paleogenomics approaches and cutting-edge methods development to leverage longitudinal sequence data of both ancient host and ancient pathogen sequence data to address coevolution with a temporal resolution that has not previously been reached.

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Title: From self-reproduction to evolution in the RNA world

Abstract: Is evolution possible in the absence of template-based replication?

Evolution, in life as we know it, relies on the copying of DNA with errors, providing the basis of reproduction with heredity and variation. Likewise, in the hypothetical RNA world, copying of RNA with errors is thought to have played the same role. However, the high complexity of RNA polymerases suggests that replication must have been preceded by reproduction and evolution based on simpler catalysts. Reproduction can occur by autocatalytic synthesis of single ribozyme species from RNA fragments, or by collective autocatalysis of multiple ribozyme species, all denoted AutoCatalytic Systems (ACS). For ACS to evolve in an open-ended way, theory indicates that there must exist a large diversity of such ACS throughout the sequence space. Further conditions for evolution are that ACS must amplify within compartments (enabling reproduction with heredity), propagate to other compartments as a function of their differential amplification (selection), and that rare events trigger the appearance of novel ACS (variation).

We aim to test the hypothesis that RNA reproduction based on ACS is widespread in the sequence space, and from this diversity, demonstrate that evolution in ACS is indeed possible.

For this, we will generate a large landscape of self-reproducing molecules using the natural group I intron family as an input for statistical inference methods. Self-reproducers will be constructed by fragmentation of these ribozymes by generalizing the strategy formerly applied to a ribozyme from the Azoarcus bacterium, and developing innovative in vitro screening methods with droplet microfluidics. We will show how the autocatalytic dynamics of individual self-reproducers, and their propensity to catalyse the formation of other self-reproducers or self-reproducing networks, allow to implement the properties of reproduction with heredity and variation underlying evolution.

Evolution will be tested in bulk and in compartmentalized populations, by submitting ACS to cycles of incubation and propagation. Finally, the probability of emergence of evolution in ACS and its open-ended character will be assessed based on the density of functional reproducers and their evolutionary accessibility in the sequence space.

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Title: The dynamics of information flow in a social network of mutually shading plants

Abstract: Social interactions between individuals lead to emergent collective behavior, whereby locally acquired information yields decentralized collective decisions, implying a flow of information within a social network. However, since social interactions are generally not directly accessible, and network structure changes according to different social and ecological contexts, this flow is difficult to observe and little is known about the principles governing its dynamics. We hypothesize that network structure shapes the dynamics of information flow and its characteristics, which can adapt according to the social context. Based on this concept, we suggest a novel and experimentally tractable system of self-organized crowded plants interacting via mutual shading while competing for light. This system is amenable to a social network analysis where nodes, representing individual plants, are connected via edges representing unidirectional and deleterious shading interactions which can be observed. The flow of information is represented by cascades of growth-driven morphological changes in neighboring plants as a response to shading manipulations, where the kinematics of individual responses to shade are described mathematically. We aim to uncover the dependence of information flow on network structure by considering mathematical properties of observed flow dynamics resulting from perturbations of the network structure, and interpret these results in terms of ecological and selective consequences. Capitalizing on the advantages of this unorthodox model system, we will tackle this goal through the following complementary and interdisciplinary lines of investigations: (i) run experiments on the system of mutually shading plants, designed to probe the dynamics of information flow; (ii) analyze experimental data in terms of social networks, (iii) combine simulations and experiments to analyze mathematical properties of observed flow dynamics as a function of network structure. These steps will allow us to interpret ecological aspects of social networks in terms of efficiency of information flow and network structure. This work will impact the fields of social ecology, plant science, and collective behavior, providing a quantitative understanding of dynamics of social information as never done before, and suggesting solutions for the optimization of agricultural crop stands.

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Title: Creating a symphony from noise: stochastic and coordinated regulation of stem cells in embryogenesis

Abstract: Embryos develop precisely at the multicellular level. Yet, stochasticity at the single cell level generates local variability in behaviors (e.g. in cell division, cell positioning, and gene expression). How is this apparent contradiction resolved? Do embryos compensate or possibly exploit local variability to adjust or correct patterns?

In mammalian embryos, the first developmental axis forms in the blastocyst when the outer trophoblasts (the future placenta) form a globe with an axis of proliferation/differentiation originating from the cluster of inner embryonic cells (the future embryo).

Here, we will investigate the principles underlying axis formation through a unique combination of stem cell-based embryology, quantitative imaging of the phenome of trophoblasts, and computational and statistical modeling. Using a novel blastocyst model formed solely with stem cells (Nicolas Rivron, The Netherlands), we will tune the embryonic signals and richly quantify the impact on trophoblast phenotypes, and their variability and precision in space (Shantanu Singh, USA), to model cells' coordination during axis formation (Kyogo Kawaguchi, Japan).

This unique synergy will reveal how individual stem cells resolve the contrasting forces of single cell variability and multicellular guidance (e.g. embryonic inductions, neighbor coupling), to adjust and achieve a level of precision during the generation of an axis.

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Title: Paradoxical responses of immune systems at the tipping point

Abstract: Basic knowledge is lacking to understand immunological tipping points, where the same stimulus can cause paradoxical immune responses. For example, immune activation after injury or infection is required for downstream tissue repair and pathogen removal, but may also cause tissue damage; immune tolerance or rejection may occur after similar type of tissue transplant, but it is difficult to predict the outcome a priori; inflammation is needed to form blood vessels connecting mother and embryo upon implantation, but too much inflammation can lead to immune disorders during pregnancy. In contrast to these critical clinical complications caused by immune overactivation, cancer cells can bias the tipping point towards the opposite direction to suppress the immune response, but the underlying mechanism still remains unclear. Driven by a series of recent breakthroughs made in our laboratories, we propose to establish planarian flatworms as a novel ideal model to delineate these paradoxical effects: with their simple immune system, we will quantify and manipulate immune cell behaviors both directly in live animals and in engineered ex vivo reconstituted cell systems; with their unsurpassed regenerative ability, we will test the response of immune cells to immunological challenges during allogeneic tissue transplantation. Integrating quantitative experiments, genetic manipulation, and mathematical modeling, we will connect gene functions, cell feedback circuits, and organismal phenotypes, to provide for the first time a multi-scale systems-level mechanism by which the immunological tipping point balances the system sensitivity and robustness. This mechanistic and predictive understanding will also allow synthetically rebuilding immune cell circuits ex vivo for bioengineering and therapeutic purposes. Our work will establish a mechanistic footing to understand more complex immune systems, and can help optimize outcomes in cancer immunotherapy, tissue transplantation, autoimmune diseases, and pregnancy disorders. This work is only achievable through a unique international collaboration that bridge several distinct fields: cellular immunology, functional genomics, and quantitative biology.