Presented in order listed

In vivo dynamic equilibration of multicellular motion during olfactory neurogenesis
Vijay Warrier, University of Illinois Chicago

Olfactory neurogenesis is a fascinating research context because it involves the contributions of multiple stem cell populations (the neural crest and ectodermal placode) to sensory neurons that retain regenerative capacity into maturity. To understand the relationships between these populations, we need to characterize complex fourdimensional phenotypes in vivo. We take advantage of the zebrafish embryonic model system and new imaging technologies to accurately track cranial neural crest and ectodermal placode-derived stem cell migration in vivo for hundreds of cells during olfactory organ patterning. These data have allowed us to identify, at a system-wide level, patterns of multicellular relationships between individual progenitor cells and cell lineages that form olfactory sensory neurons. We have focused on the posterior-to-anterior ingression of neural crest cells into the olfactory epithelium by considering displacement time series of both types of cells and analyzing these data with methods derived from econometrics, namely volatility analysis and cointegration. Our analyses demonstrate that cranial neural crest cells migrating into the olfactory epithelium segregate, based on behavioral characteristics, into two distinct, previously unidentified subtypes that we have termed 'trend' and 'dispersed' lineages. Furthermore, we show that neural crest and placodal progenitor migration and intercalation are coordinated by at least two novel types of collective behavior. The first is a dynamic stochastic equilibration of the average mean separation between and within sets of placode and neural crest lineages, i.e. the lineages behave as if they are 'elastically' tethered. The second behavior, 'spatiotemporal exclusion', involves a striking lack of trajectory overlap between dispersed neural crest lineages and placodal lineages. Together, our results reveal a highly dynamic interplay between different progenitor cell types during the formation of the olfactory system and demonstrate how unbiased statistical methods from econometrics have utility in the characterization of complex developmental patterns.

Quantitative Morphometrics Reveals Multiple Roles for Notch Signaling in Olfactory Neurogenesis
Sriivatsan G. Rajan, University of Illinois at Chicago

The zebrafish olfactory epithelium (OE) is a complex tissue composed of two main types of olfactory sensory neurons, microvillous (mOSNs) and ciliated (cOSNs), that detect a wide range of odors and other signals. We and others have previously shown that zebrafish mOSNs and cOSNs come from two different stem cell populations, namely neural crest stem cells (NCCs) and placodal progenitors, respectively. In the murine OE, the Notch signaling receptor *notch3* has been shown to be expressed in the developing neuroepithelium, but the molecular mechanisms through which Notch signaling might regulate cellular processes such as migration, differentiation, and axon pathfinding in vertebrate OSNs remain largely unexplored. Therefore, we performed RNA *in situ* hybridization for Notch signaling receptors in zebrafish and found that *notch1a*, *notch1b*, and *notch3* were expressed in the OE in discrete patterns that varied dynamically during early stages of olfactory neurogenesis. Next, we inhibited Notch signaling using both a chemical inhibitor and a heat shock-inducible genetic knockdown line during specific time windows and performed time-lapse imaging in live embryos at high spatiotemporal resolution. We analyzed our imaging data using quantitative morphometrics to measure parameters such as total volume of cell clusters and distance between OSN axon terminals. Our analysis revealed that the absence of Notch signaling led to significant variations in the numbers of both OSN subtypes, changes in the total volume of cOSNs, and abnormalities in the axon projections of a specific subset of OSNs. Taken together, our data suggest that Notch signaling plays spatiotemporally-critical roles in regulating OSN differentiation and subsequent axon guidance. Now, we are validating our results with tissue-specific knockdown of Notch signaling and developing new genetic tools to selectively inhibit Notch receptors *in vivo* so as to understand at a finer level how Notch signaling may contribute to olfactory neurogenesis.

Quantitating the in vivo relationship between Notch signaling and the protein Olfactin in the developing olfactory system
Joseph N. Lombardo, University of Illinois at Chicago

Development of the vertebrate olfactory system is mediated by a complex set of biological processes spanning multiple tissues and cell types. In zebrafish, the olfactory epithelium (OE) contains two main types of olfactory sensory neurons—ciliated (cOSNs) and microvillous (mOSNs)—which project axons to the olfactory bulb (OB). A previous protein trapping screen yielded a transgenic zebrafish line with a currently unidentified protein, which we have named Olfactin, fused to a Citrine fluorophore. Expression of this fluorescent fusion protein, Olfactin-Citrine (Olf-C), occurs in the OE and OB at the earliest stages of their formation, and therefore, we hypothesize that Olf-C may play a critical role in olfactory development. Through in vivo confocal microscopy and the quantitative measurement of fluorescence intensities that directly represent Olf-C protein concentrations, we obtain linearly-scaled readouts of Olf-C levels in the developing olfactory system with high spatiotemporal resolution. We can use these readouts to investigate which signaling pathways modulate Olf-C protein levels and subcellular localization. Notch signaling is a highly conserved pathway known to play critical roles in cell fate and differentiation in multiple tissues. Based on the expression pattern of Notch receptors in the olfactory system, we blocked Notch signaling in vivo with the ɣ-secretase inhibitor DAPT from 24 to 31 hours post-fertilization (hpf), a significant timeframe for OSN differentiation, and imaged embryos from 36 to 48 hpf. We found that inhibition of Notch signaling increased the average level of Olf-C protein per cell in the OE while not affecting the overall number of Olf-C+ cells. Further quantitation revealed that the distribution of Olf-C concentrations was differentially affected in cOSNs and non-cOSN cells. To validate these results, we induced overexpression of Notch signaling via the heat shock transgenic zebrafish line hsp70:NICD at 22 hpf and imaged embryos from 33 to 48 hpf. Preliminary data suggest that increased Notch signaling initially reduced Olf-C in the cOSNs, consistent with the previous effects of Notch signaling inhibition. However, by 48 hpf, Olf-C levels in cOSNs of heat-shocked transgenic embryos had surpassed that of their control siblings, perhaps suggesting the presence of a dynamic reequilibration system in the developing olfactory system. Future investigation will clarify the modulatory effects of Notch signaling on Olf-C protein levels and localization in OSNs, in addition to directly investigating Olfactin's roles in olfactory development.

Neuroblastoma Differentiation In Vivo Excludes Cranial Tumors
Ankur Saxena, University of Illinois at Chicago

Neuroblastoma (NB), the most common cancer in the first year of life, is thought to arise from the neural crest (NC)-derived sympathoadrenal lineage and is found almost exclusively in the trunk. To understand why an early-onset cancer would have such a specific localization, we xenotransplanted human NB cells into discrete NC streams in developing zebrafish embryos. We demonstrate quantitatively that human NB cells previously shown to be incapable of differentiation remain undifferentiated when comigrating posteriorly with native NC cells but, upon comigration into the head, readily differentiate into neurons and exhibit decreased survival. Furthermore, we demonstrate that this in vivo differentiation requires retinoic acid, a commonly used but poorly understood NB treatment, as well as the scaffolding protein intersectin-1. We also establish the ability of retinoic acid to partially rescue differentiation in intersectin-1's absence. Our quantitative findings suggest a microenvironment-mediated explanation for NB's trunk-biased localization in young patients, highlight the potential of neuronal differentiation in promoting NB resolution, and suggest that the paradigm of a sympathoadrenal-derived cancer should be reevaluated to consider NB an NC-derived cancer with limited in vivo survivability.

Decipher the Gene Regulatory Mechanisms of Somatic Tissue Homeostasis
Xiaomin Bao, Northwestern University

An average human body loses 50-70 billion cells everyday due to wear and tear. To maintain tissue integrity, adult stem cells must precisely balance their fate choices between proliferate and differentiation. The gene regulatory network governing proliferation versus differentiation remains incompletely understood. My group leverages human epidermal tissue as the primary research model. We have previously identified the BAF chromatin remodeling complex and the PRMT1 protein arginine methyltransferase as key epigenomic regulators controlling epidermal tissue homeostasis. We have further identified novel transcription regulators that controls gene expression beyond transcription initiation. The long-term goal of my research is to discover novel molecular mechanisms underlying human tissue homeostasis, providing new ideas for disease therapies.

A new tool for anaerobic fluorescence imaging of microbiomes
Hannah E. Chia, University of Michigan

While live-cell fluorescence microscopy has flourished with the use of fluorescent proteins like GFP, GFP-like proteins are oxygen-dependent to produce fluorescence and cannot be used to study anaerobic systems such as the gut microbiome. To address this crucial limitation, we have used the fatty-acid-binding fluorescent protein, UnaG, to implement anaerobic live-cell imaging of the gut bacterium Bacteroides thetatiotaomicron (B. theta). We have assessed the efficiency of fluorescent cytosolic and membrane bound UnaG labeling in B. theta in mono-culture and in mixed cultures of B. theta and Ruminococcus bromii (R. bromii) through live-cell anaerobic fluorescence microscopy and fluorescence assisted cell sorting. UnaG may be further engineered and utilized to become a more robust tool capable of anaerobic fluorescence labeling to study complex interactions between species of a polymicrobial community.

Comparing Fluorescent Staining versus Digital Staining as a Method for Living Cell Discovery
J. Coleman, IL Science + Technology Park

Improve the study of *in vivo* cell biology by using non-invasive holographic tomographic microscopy to analyze the data of cell types, organelles, and dynamic processes such as cell division, cell morphology in biochemical assays for drug discovery screening, and cell death. Specifically, the use of fluorescent staining versus digital staining as a method for cell discovery will be compared. A live cell is transparent and offers no contrast for imaging in classical optical microscopy. Hence, chemical markers are used to provide contrast between cells and their subsystems. Traditional fluorescence microscopy is invasive, marker-dependent, and has limited end points. An intense high power laser is required to excite an exogenous fluorescent marker added to live cells, which is incompatible with normal physiological conditions and causes bleaching, phototoxicity, and limits observation time. On the other hand, digital staining used in non-invasive 3D live cell imaging involves no bleaching, no phototoxicity, and is marker free. A low intensity laser is used to scan a cell sample at the rate of 1.5 second per 3D image. Digital staining allows you to observe and identify cells based on their refractive index (an inherent natural property) for quantitative analysis via a color code to generate a 3D reconstruction of a cell. Various live cell samples will be studied using fluorescence staining and digital staining to compare and contrast the effectiveness of fluorescence microscopy versus holotomographic microscopy.

Shape Dependent Motility During the Establishment of Tissue Structure
John Devany, The University of Chicago

Mature epithelial tissues have distinct cellular architecture, which is maintained despite externally applied forces, wounding, and cell division or death. Here we investigate how a model tissue develops and maintains cellular structure by quantifying single cell dynamics and cell shape in newly formed MDCK monolayers. Over time cells in the monolayer become increasingly hexagonal and arrest at a final structure resembling a mature epithelium. Throughout this process we observe glassy dynamics controlled by cell shape, as predicted by vertex models. Varying substrate stiffness causes monolayers to form and evolve with different cell density, but a similar relationship between cell shape and dynamics. This suggests the changes in cell density often observed in tissue development may not directly impact cell motility. We find that inhibiting regulators of the actin cytoskeleton cause monolayers to arrest with elongated cell shapes. Interestingly, across a diverse set of conditions we find a relationship between the final cell shapes and velocity correlation length which we explore in vertex models by including cell alignment coupling. Our results demonstrate that multicellular coordination of motility affects the regulation of cell shape and determination of final tissue structure.

Insect embryos begin as large multinucleate cells: insights into tissue organization and evolution over eight orders of magnitude
Seth Donoughe, The University of Chicago

In most insect species, development begins as a syncytium: that is, many nuclei divide and move within the single shared cytoplasm of the egg. These nuclei self-organize into a uniform layer that forms the initial tissue of the insect embryo. The mechanistic basis of the nucleus movements and their spatial organization is a mystery. We use lightsheet microscopy to live-image transgenic embryos of the cricket *Gryllus bimaculatus* with high temporal resolution. We automatically detect and track nuclei, and then quantitatively characterize nuclear divisions and movements of thousands of nuclei in 3D space for up to 7 hours at a time. These data, combined with physical manipulations to the egg, have enabled us to generate a simple geometric model of local crowding that explains embryo-wide patterns of nucleus movement. We have also compiled a dataset of the sizes and shapes of the eggs from 6000+ species of insects from all major clades. We've found that syncytial eggs span more than eight orders of magnitude in volume, and over the course of evolutionary history, there have been dozens of independent increases and decreases in the size of the egg. Thus, evolution has provided us the data from a natural experiment on how basic cell biological processes scale across a vast range of sizes. We use these data to test numerous standing hypotheses about egg evolution.

Diffusion vs. direct transport in the precision of morphogen readout
Sean Fancher, Purdue University

Morphogen profiles allow cells to determine their position within a developing organism, but there are multiple mechanisms by which these profiles form, and in some cases the mechanism is still not agreed upon. Here we derive fundamental limits to the precision of morphogen concentration sensing for two canonical mechanisms: the diffusion of morphogen through extracellular space and the direct transport of morphogen from source cell to target cell, e.g. via cytonemes. We find that direct transport establishes a morphogen profile without adding extrinsic noise. Despite this advantage, we find that for sufficiently large values of population size and profile length, the diffusion mechanism is many times more precise due to a higher refresh rate of morphogen molecules. We compare our predictions with data from a wide variety of morphogens in developing organisms.

Interphase chromatin as a self-returning random walk
Kai Huang, Northwestern University

We introduce a self-returning random walk to describe the structure of interphase chromatin. Based on a simple folding algorithm, our de novo model unifies the high contact frequency discovered by genomic techniques, and the high structural heterogeneity revealed by imaging techniques, which two chromatin properties we theoretically prove to be irreconcilable within a fractal polymer framework. Our model provides a holistic view of chromatin folding, in which the topologically associated domains are liquid-tree-like structures, linked and isolated by stretched out, transcriptionally active DNA to form a secondary structure of chromatin that further folds into a "3D forest" under confinement. The model pivots a wide array of experimental observations and suggests the existence of a universal chromatin folding principle. Based on a global folding parameter, the model reveals a unique structure-function relation of chromatin, which is abnormal from a polymer point of view but explains some experimental observations of how chromatin responses to stress.

The Self-tuned Sensitivity of Circadian Clocks
Kabir B Husain, University of Chicago

Living organisms make unreliable observations of their environment while also making unreliable internal predictions of what their environment should be. The theory of Kalman filtering suggests that predictions should be updated by measurements using a time-varying environmental sensitivity that reflects the estimated unreliability of these quantities. We show that the circadian clock in S. elongatus can naturally show such self-tuned environmental sensitivity by analyzing the feedback coupling between clock and metabolism quantified in recent experiments. The metabolic coupling detects mismatch between clock predictions and environmental light conditions and can temporarily raise the clock's sensitivity to light changes, allowing faster entrainment in case of mismatch. We also analyze analogous behavior in recent experiments on switching between slow and fast osmotic stress response pathways in yeast. In both cases, Kalman-like strategies allow an organism to naturally switch from states of low to high sensitivity under stress. Our work suggests experiments that probe the history-dependence of environmental sensitivity in biophysical sensing mechanisms.

Regulation of Motions of Myosin Motors in the Actin Cortex
Wonyeong Jung, Purdue University

Active transport driven by molecular motors in the cytoskeleton plays an important role in various cellular processes. It has been hypothesized that motions of myosin motors in the cortex are determined by the architecture of the cortex. However, the effects of dynamic, force-dependent behaviors of cytoskeletal components on myosin motors remain elusive despite their potential importance. In this study, we employed an agent-based computational model to study motions of myosin in the cell cortex. The model accounts for possible governing factors, including force-dependent walking of motors and the turnover of cross-linkers and F-actin. We found that motions of motors can be suppressed due to three reasons. Motors can slow down significantly either by local force generation or global force transmission between motors. It is also possible F-actin aggregation prevents motors from consistently walking. However, F-actin turnover can recover motor motility in all three cases by inducing force relaxation on motors and cross-linkers. Our results shed light on how myosin motions are regulated by many factors in vivo.

Regulation of Pulsed Contraction of Actomyosin Networks
Jing Li, Purdue University

Actomyosin contractility regulates various biological processes including cell migration and cytokinesis. Cell cortex underlying a membrane, which is a representative actomyosin network in eukaryote cells, exhibits dynamic contractile behaviors. Interestingly, the cell cortex shows reversible aggregation of actin and myosin called pulsatile contraction in diverse cellular phenomena, commonly associated with the spatiotemporal expression of a Rho GTPase: RhoA. While contractile behaviors of actomyosin machinery have been studied extensively in several in vitro experiments and computational studies, none of them successfully reproduced pulsed contraction observed in vivo. In this study, we employed an agent-based computational model based on Brownian dynamics to identify critical factors facilitating the pulsatile contraction of actomyosin networks. We reproduced the pulsed contraction only with the mechanical and dynamic behaviors of cytoskeletal components. We found that clusters with physiologically relevant size and duration can appear in the presence of both F-actin turnover and angle-dependent F-actin severing resulting from buckling induced by motor activities. We concluded that the strong pulsatile contraction of actomyosin networks only occurs when there is subtle balance between force generation from motors, global force relaxation via actin turnover, and local force relaxation via angle-dependent actin severing. Our study sheds light on the underestimated significance of F-actin dynamics for the pulsed contraction during various physiological processes.

Pre-Knowledge Based Lasso for Gaussian Graphical Models
Keren Li, Northwestern University

Structure learning in graphical models has drawn considerable attention in many fields, such as computational biology, protein networks, and social network analysis. In such fields there often exists annotated database for interactions between nodes, which is useful but often incomplete or error-prone. The real data collected however frequently faces the curse of small sample size and high imensionality, lacking the power to infer the complete graph structure in a blind way. How to combine the real data and existing knowledge for discovery of graph structures becomes a challenging problem. We propose a pre-knowledge based lasso for the Gaussian graphical model, using the known partial structure to configure initial weights of regularization parameter for a pathwise regression. Simulation studies show that it outperforms original lasso in terms of either true positive rates or true negative rates for $p > n$ case. This method can be implemented to other lasso-family members, such as SCAD, elastic net and adaptive lasso, etc.

Quantification Analysis in Site-Directed Mutagenesis of *N*-glycosyltransferases for Peptide Substrate Library Preference
Liang Lin, Northwestern University

Mutagenesis is the basic method to produce enzymes for novel substrates. Many methods have been developed to fast screen and pick up positive single clones. However, there still lacks of a quantification method to analyze the quality of the mutagenesis and guide the mutagenesis. Here, to produce *N*-glycosyltransferase (NGT) mutants for novel peptide substrates, we synthesized a total peptide substrate library $X_{-1}NX_{+1}T$ to screen each mutant. Every potential substrate binding residues were mutated to the other 19 amino acids pool, individually, and every site mutants pool were screened with whole substrate library. The preference change at X_{-1} and X_{+1}, the relative k_{cat}/K_M, and the relative percentage library preference were quantificationally analyzed, and the binding residues for X_{-1} and X_{+1} were selected, along with other substrate effective residues. We set up thresholds for strong, week and non-effective. Single amino acid mutagenesis of effective residues was also quantificationally analyzed and some rules were summarized to direct future mutagenesis.

Growth Control of *Drosophila* Wings
Andrew Liu, Northwestern University

One fundamental aspect of developmental biology is growth control – how growth rate and final organ size is determined. The *Drosophila* wing imaginal disc is a well-established model system, where growth is spatially homogenous and exponential during the second and early third instars. Previous research suggested that, in the wing pouch, the gradient of Dachsous (Ds) and Four-Jointed (Fj) within the Fat signaling pathway may regulate growth. Specifically, it is the juxtaposition of high and low Ds/Fj in neighboring cells that drives proliferation. Therefore, a young, small disc with a steep gradient will continue to proliferate while an older, large disc with a shallow gradient will not proliferate further. My first aim is to quantify and manipulate the global gradients of Ds and Fj using temperature, nutrition, and gender differences. Furthermore, *ex vivo* live-imaging of wing disc explants has been successfully implemented to support growth similar to *in vivo* conditions. I seek to combine live imaging with microfluidic devices to manipulate local gradients and assay the changes in growth control.

Kinetic driving that converts a foe into a friend -- inspirations from gene expression
Zhiyue Lu, The University of Chicago

In gene expression regulation, repressors and activators compete to bind with the gene's regulatory element, and the chance of activators binding with the regulatory element dictates the activation level of the gene. In a thermal equilibrium regime, this chance of binding is well determined by the concentrations of activators and repressors as well as their binding free energies to the regulatory element -- a high concentration of repressor will result in a low chance of activator binding. However, inspired by the experimental observations of cooperativity between activators and repressors, we propose a non-equilibrium dynamics model to describe the gene regulatory dynamics when the concentration of the repressor changes rapidly over time. We demonstrate a minimal Markov model where a rapidly changing level of repressor significantly increases the level of activation beyond predicted by equilibrium theory and effectively enhance the activator. Our model introduced an internal degree of freedom of the regulatory element, and its kinetic barrier allows the system to harnesses the chemical work done by the rapidly changing concentration of the repressor and use it to boost the level of gene activation beyond the equilibrium prediction.

Higher order interaction inhibits bacterial invasion of a producer-predator microbial community
Harry Mickalide, University of Illinois Urbana-Champaign

It is important that microbial communities resist invasion in order to maintain biodiversity, stabilize industrial bioreactors, and preserve human microbiome health. It is widely believed that the more diverse a microbial community is, the more resistant to invasion it will be, and that this increased invasion resistance arises from a niche complementarity effect: more diverse communities consume a greater range of resources and thus eliminate niches for would-be invaders. Here we show that in a community of the algae *Chlamydomonas reinhardtii* (producer) and the ciliate *Tetrahymena thermophila* (predator), invasions by the bacteria *Escherichia coli* fail even when there is a niche of consumable resources available to the bacteria. In contrast, bacteria successfully invade communities of algae or ciliates alone. We attribute the invasion resistance of the algae-ciliate community to a higher-order (3-way) interaction: the algae inhibits the bacteria's ability to aggregate which leaves the bacteria vulnerable to the ciliate's predation. This method of invasion resistance requires both the algae and the ciliate to be present and thus provides an example of diversity leading to invasion resistance due to a higher-order interaction rather than niche complementarity.

Multistationarity in Biochemical Reaction Networks
Maya Mincheva, Northern Illinois University

Multistationarity is defined as the existence of several positive equilibria of an ordinary differential equations model. Multistationarity is a required property of biological switches-reaction networks that govern important cellular functions, such as cell differentiation and cell death. This is the case, because biological switches are modeled by differential equations systems whose solutions can approach different stable equilibria depending on the initial conditions. Many differential equations models of reaction networks are known to be multistationary for particular parameter values. For multistationarity to be robust, a reaction network has to be multistationary in some open region of parameter space. For a model of the double phosphorylation network, we have obtained simple parametric inequalities that identify multistationary regions in parameter space.

Coupled Differentiation and Grain Boundary Formation in the Zebrafish Cone Mosaic
Hayden S. Nunley, University of Michigan

In zebrafish retinae, cone photoreceptor cells self-organize by subtype into a crystalline lattice called the cone mosaic. This two-dimensional lattice lies on the surface of the retinal hemisphere. As the lattice grows from the rim, topological defects, called Y-junctions, must be inserted to maintain constant cell spacing. Y-junctions form grain boundaries, similar to those in physical crystals on curved surfaces. By tracking UV cone motion, we rule out Y-junction motion after initial mosaic formation as a mechanism for grain boundary formation. We then demonstrate that a mechanical model of cone mosaic development naturally generates grain boundaries during initial mosaic formation.

Calmodulin-dependent phosphatase exclusion stabilizes CaMKII auto-phosphorylation
Matthew Pharris, Purdue University

Calcium/calmodulin-dependent protein kinase II (CaMKII) is essential to learning and memory formation. CaMKII is a 12-subunit holoenzyme, with subunits arranged in two directly-apposed, radially symmetric rings. When two neighboring subunits are each activated by calmodulin, one subunit may auto-phosphorylate the other, resulting in a conformational change that preserves the subunit's activation following calmodulin dissociation. The mechanisms regulating CaMKII auto-phosphorylation remain unclear in-part due to the combinatorial explosion that arises when modeling CaMKII by conventional mass-action methods. To overcome combinatorial explosion without necessitating a loss in biological accuracy, we use a rule-based approach with the spatial-stochastic simulator MCell. With our rule-based MCell model, we present the most complete model of the CaMKII holoenzyme to-date and identify a new mechanism regulating auto-phosphorylation. Specifically, our results suggest that calmodulin not only activates CaMKII but may also preserve CaMKII auto-phosphorylation by structurally excluding phosphatase binding.

Quantitative Morphometrics Reveals Multiple Roles for Notch Signaling in Olfactory Neurogenesis
Sriivatsan G. Rajan, University of Illinois at Chicago

The zebrafish olfactory epithelium (OE) is a complex tissue composed of two main types of olfactory sensory neurons, microvillous (mOSNs) and ciliated (cOSNs), that detect a wide range of odors and other signals. We and others have previously shown that zebrafish mOSNs and cOSNs come from two different stem cell populations, namely neural crest stem cells (NCCs) and placodal progenitors, respectively. In the murine OE, the Notch signaling receptor *notch3* has been shown to be expressed in the developing neuroepithelium, but the molecular mechanisms through which Notch signaling might regulate cellular processes such as migration, differentiation, and axon pathfinding in vertebrate OSNs remain largely unexplored. Therefore, we performed RNA *in situ* hybridization for Notch signaling receptors in zebrafish and found that *notch1a*, *notch1b*, and *notch3* were expressed in the OE in discrete patterns that varied dynamically during early stages of olfactory neurogenesis. Next, we inhibited Notch signaling using both a chemical inhibitor and a heat shock-inducible genetic knockdown line during specific time windows and performed time-lapse imaging in live embryos at high spatiotemporal resolution. We analyzed our imaging data using quantitative morphometrics to measure parameters such as total volume of cell clusters and distance between OSN axon terminals. Our analysis revealed that the absence of Notch signaling led to significant variations in the numbers of both OSN subtypes, changes in the total volume of cOSNs, and abnormalities in the axon projections of a specific subset of OSNs. Taken together, our data suggest that Notch signaling plays spatiotemporally-critical roles in regulating OSN differentiation and subsequent axon guidance. Now, we are validating our results with tissue-specific knockdown of Notch signaling and developing new genetic tools to selectively inhibit Notch receptors *in vivo* so as to understand at a finer level how Notch signaling may contribute to olfactory neurogenesis.

Computational Categorization of Immune Status and Biomarker Expression in Melanoma Patients for Enhancing Prediction of Checkpoint Blockade Therapy Response
Ashmitha Rajendran, Northwestern University

Immunotherapy has revolutionized cancer treatment by reinstating the patient's immune response against cancers and reducing the immune evasion phenotype. Immune checkpoint blockade therapy (ICBT), in particular, involving monoclonal antibody (mAb) inhibitors for immune regulators CTLA-4 and PD-1 have been effective at restoring anti-tumor immunity, leading to durable and long-term responses. Although ICBT has improved tumor regression, a majority of patients fail to develop an anti-tumor immune response and can also experience a wide variety of immune related side effects (e.g. neurological, dermatological, or pulmonary toxicities), warranting subsequent immunosuppressive therapy. Increasing the response rate to ICBT and preventing unnecessary side effects requires further classification of the melanoma tumor microenvironment and correlation with expressed biomolecular factors that predict therapy response. This project will explore the hypothesis that understanding associations between pre-existing immune-related tumor microenvironments and ICBT biomarkers can provide methods to use these criteria for patient selection. Using computational tools for clustering and analysis of integrative high-throughput genomic data from the cancer genome atlas (TCGA), the results will define predictive relationships between immune related subtypes of melanoma and biomarker expression patterns that correlate with ICBT response rates. These unbiased analyses will identify new biomarker and immunologically-defined melanoma tumor subtypes that will better predict ICBT efficacy. This work will improve the selection of melanoma patients for ICBTs and develop analysis tools and methodological improvements that can be translated to other cancers.

Large-scale investigation of the reasons why potentially important genes are ignored
Thomas Stoeger, Northwestern University

Biomedical research has been previously reported to primarily focus on a minority of all known genes. Here, we demonstrate that these differences in attention can be explained, to a large extent, exclusively from a small set of identifiable chemical, physical, and biological properties of genes. Together with knowledge about homologous genes from model organisms, these features allow us to accurately predict the number of publications on individual human genes, the year of their first report, the levels of funding awarded by the National Institutes of Health (NIH), and the development of drugs against disease-associated genes. By explicitly identifying the reasons for gene-specific bias and performing a meta-analysis of existing computational and experimental knowledge bases, we describe gene-specific strategies for the identification of important but hitherto ignored genes that can open novel directions for future investigation.

Interpreting morphogen gradients in cochlea development
Matthew J. Thompson, Purdue University

Quantitative, systems-level characterization of cochlea development is needed to gain further significant insight into how the functionally necessary spatial organization arises in this intricately patterned sensory organ. The organ of Corti contains a single row of inner hair cells, three rows of outer hair cells, and supporting cells arranged with a positional accuracy less than one cell diameter. This pattern emerges from a homogeneous field of prosensory epithelium. Major morphogenetic pathways active during the critical period for cell specification in the organ of Corti have been identified, including Bmp, Wnt, Notch, Fgf, and Shh. Preliminary data quantitatively addressing gradient interpretation within the prosensory epithelium of pSmad, an indicator of Bmp4 activity, and Sox2, a downstream integrated output of the combined network, will be presented.

Modeling Cell Polarization in Fission Yeast
Bin Xu, University of Notre Dame

Regulation of polarized cell growth is essential for many cellular processes, including spatial coordination of cell morphology changes during growth and division. We present a mathematical model of the core mechanism responsible for the regulation of polarized growth dynamics by the small GTPase Cdc42. The model is based on the competition of growth zones of Cdc42 localized at the cell tips for a common substrate that diffuses in the cytosol. We analyze the bifurcations in this model as the cell length increases. We find that a stable oscillation and a stable steady state can coexist, which is consistent with the experimental finding that only 50% of bipolar cells oscillate.

Reverse-Engineering Calcium Signaling in a Developing Organ
Jeremiah J. Zartman, University of Notre Dame

Organ development depends on a complex cellular signal transduction network for cells to sense and integrate information from a diverse range of chemical and mechanical cues. Signals are transduced to regulate cell processes through key integrators such as calcium ions. However, how calcium signaling contributes to organogenesis at a systems level is poorly understood. Very few tools exist to study cell signaling at millimeter length scales. Here, we present collaborative efforts to advance organ-level experimentation and quantitative analysis methods. We developed a fluidic device called the Regulated Epithelial Microenvironment Chip to modulate multiple stimuli acting on micro-organs. As a specific demonstration, we are using advanced genetic tools available in a fruit fly-based model of organ growth, the larval wing imaginal disc, to quantitatively study the regulation and function of calcium signaling dynamics occurring during organ growth *in vivo*. The system also enables live imaging of cell signaling responses. This approach enabled us to decouple the contributions of biochemical signaling and mechanical loading to calcium signaling dynamics. We discovered that mechanical stimulation of intercellular calcium waves (ICWs) depended upon the pre-loaded organ state, rather than the magnitude or duration of mechanical loading. Intercellular calcium signaling relies on calcium induced calcium release and propagation through gap junctions. These results provide evidence for a “Mechanical Stress Decay” hypothesis of calcium signaling. This hypothesis states that stimulated intercellular calcium transients provide a readout of organ size and contribute to the regulation of growth, cell mechanics and cell differentiation as the organ grows. These studies help identify strategies for spatiotemporally encoding information through calcium signaling within the multicellular context. Ultimately, advances in calcium signaling engineering can lead to novel approaches to detect a broad range of stimuli in cell-based biosensors, target cancer, and accelerate tissue regeneration.