Developing a Null Model with the Curveball Algorithm for Sampling Random Protein Networks.
Akua Biaa Adu and Keenan M.L. Mack

Poster presented by Akua Biaa Adu, Illinois College

In a normally functioning cell, protein interact by binding to each other to perform the work of the cell. Understanding the patterns of protein-protein interaction could illuminate how genetic pathways are regulated, how mutations effect complex gene networks, and how genomes evolved. Null models allow us to check observations against theoretical expectations. Previous analyses of published protein-protein interaction networks show that they exhibit patterns of degree distribution (how many nodes in the network are highly connected relative to sparsely connected) and degree correlation (whether those highly connected nodes are more likely to be connected to other highly connected nodes or sparsely connected nodes). The generation of these patterns is thought to result from the assembly mechanisms operating in real world networks. However, the current proposed assembly algorithms cannot explain the observed degree correlation in protein-protein interaction networks. This work (curve-ball algorithm; which is code written in java programming language) will allow us to build a null model by generating a representative set of random networks with a specific degree distribution and test whether our proposed assembly algorithm can generate the observed patters of degree correlation and distribution in protein interaction networks. Understanding protein-protein interaction networks is crucial for understanding cell physiology in both normal and disease states.

Notch Signaling Regulates Stem Cell Behavior During Ciliated Olfactory Neuron Differentiation
Nathan Burg, Sriivatsan G. Rajan, Jocelyn Garcia, Ankur Saxena

Poster presented by Nathan Burg, University of Illinois at Chicago
Finalist Prize for Undergraduate Research in Quantitative Biology

Ciliated olfactory sensory neurons (cOSNs) constitute a primary sensory neuron subtype in the vertebrate olfactory epithelium (OE) that is used to detect volatile odors. The Notch signaling pathway has been shown to play important roles in cell proliferation and differentiation across many developing organ systems, but the possible mechanisms and downstream pathways through which it might regulate the differentiation of cOSNs are relatively unexplored. We have discovered that multiple Notch signaling components are dynamically expressed in the OE during early development. Using both genetic and chemical Notch signaling inhibitors, we found a statistically significant increase in the number of cOSNs and a statistically significant decrease in the number of progenitors in response to inhibition of Notch signaling. Analyzing the mean fluorescence intensity of immunostaining for a progenitor cell marker revealed that the observed decrease in progenitor cell numbers upon Notch signaling inhibition occurs in a distinct subset of the total progenitor cell population. Taken together, our findings suggest that the increase in cOSN numbers upon Notch signaling inhibition may be due to the premature differentiation of progenitor cells.

Northwestern University's High Throughput Analysis Core
Curt Horvath, Sara Fernandez Dunne

Poster presented by Sara Dunne, Northwestern University

Projects involving massively parallel experiments are opening unexpected frontiers in basic science and accelerating development of new medicines. Doing them well and getting useful data analysis usually requires specialized expertise and a suite of pricey equipment. This isn't possible for many laboratories, especially in academia. Northwestern's High Throughput Analysis Lab (NU-HTA) has grown over the last decade into a premier open resource for this kind of research. We're committed to providing Chicago's life science community an affordable way to set up, run, gather data and perform analysis from tens to tens of thousands of parallel experiments using up-to-date instruments. This includes drug discovery research, biochemistry, cell and organismal biology, functional genomic screening, and synthetic genetic analysis. Proteins, nucleic acids, cells, small model organisms, and microbial strains are things we work with. Come see what we can do for you today!

The role of the basement membrane in mediating basal tissue folding
Elizabeth Falat, Jennifer Wendlick, Michael Stoneman, Valerica Raicu, Jennifer Gutzman

Poster presented by Elizabeth Falat, University of Wisconsin-Milwaukee

Morphogenesis requires proper cell shape changes and mechanical force, such as tension. Both of these regulators are known to be mediated by intracellular components, such as the actin and microtubule cytoskeletal networks; however, there is a significant gap in our understanding of how interactions between cells and their extracellular environment, specifically with the extracellular matrix (ECM), function to mediate these morphogenetic processes. The basement membrane is a specific type of ECM found along the basal surface of epithelia and is primarily composed of laminin, collagen, and proteoglycans such as agrin. This matrix was previously regarded as a static scaffold, functioning as a passive structure to mediate cell adhesion and polarity. Now, the basement membrane is emerging as a dynamic network critical for regulating mechanical forces required during morphogenesis. We are investigating this role for the basement membrane using the highly conserved zebrafish midbrain-hindbrain boundary (MHB) as a model. We have begun to elucidate the role for different laminin genes in mediating the cell shape changes required for MHB morphogenesis. Using live imaging of Laminin-111 zebrafish mutants, we found that lama1, lamb1, and lamc1 are all required for proper MHB tissue folding. In addition, we have identified a role for lama1 in mediating basal cell shape and are in the process of cell shape analysis for lamb1, lamc1 and agrn mutants. Our current experiments are utilizing two-photon laser ablation to study localized tissue tension needed to fold the MHB. Our objective is to identify if components of the basement membrane that are critical for cell shape are also contributors to the mechanical forces that are required during brain morphogenesis.

How Many Flagella is Enough? Fitness Costs of Flagella in Salmonella enterica
Joshua Franklin, Imke Spöring, Keara Grady, Yann Dufour

Poster presented by Joshua Franklin, Michigan State University

Bacteria possess a wide range of flagellar configurations, but we usually cannot explain why a species of bacteria has a given number or arrangement of flagella. It is not clear why some bacteria produce multiple flagella; uniflagellate bacteria swim very well in aqueous environments, so why spend energy making additional flagella? To understand the evolutionary forces shaping flagellar expression, we needed to quantify the fitness tradeoffs involved. We used a Salmonella enterica strain in which the master regulator of flagellar expression, flhDC, is under the control of a tetracycline-inducible promoter. This strain also has a flgE(S171C) mutation, which allowed us to fluorescently label flagellar hooks and count flagella number as a function of inducer concentration. Using a custom cell tracking setup, we directly correlated flagella number with swimming behavior for individual cells. To examine the benefit of flagella in a more complex environment, we measured colony size on soft agar. Finally, we measured growth rates across a range of inducer concentrations to quantify the metabolic cost of making flagella. Given the observed flagella number and growth rate at each inducer concentration, we found that each additional flagellum reduced growth rate by approximately 1%. When tracking in low-viscosity liquids, we found no correlation between flagella number and diffusion coefficient. However, we saw a clear positive relationship between flagella number and colony size on soft agar. Taken together, our results suggest that peritrichously flagellated bacteria pay a significant cost to produce flagella, which provide almost no benefit in low-viscosity liquids. Instead, the primary benefit of peritrichous flagella may be enhanced movement through porous and viscous environments, such as soil or mucus. Future experiments will examine the exact mechanism by which multiple flagella improve swimming in these environments.

Progression of a mechanical waves initiates the self-organization of multicellular rosettes in the Drosophila compound eye
Kevin D Gallagher, Madhav Mani, Richard W Carthew

Poster presented by Kevin Gallagher, Northwestern University

Spatially extended patterns of cell morphology and identity cannot be genetically encoded. Instead, such patterns must emerge dynamically during development. In the Drosophila compound eye, ommatidia (unit-eyes) self-organize into 5-cell rosettes while simultaneously differentiating into photoreceptor subtypes. How

gene expression and morphology interplay during this process is not well understood. Therefore, we created a technique to ex vivo culture the eye imaginal disc for up to 16 hours. This technique allows real-time visualization of morphological features and gene regulation through fluorescence-based reporters. Patterning of the eye initiates with a mechanical wave, called the morphogenetic furrow (MF), that propagates across the eye disc. The MF imparts patterning on two spatial scales: (1) on the local scale, multicellular rosettes bud off the posterior edge of the MF, like water droplets beading up on a clothes line and (2) on the tissue scale, nascent ommatidia emerge from the MF in a precise hexagonal array. Observations from fixed tissue lead to the inference that the MF propagates through the tissue like a compression wave – e.g. the topology of cells is not significantly altered as they pass through the MF. However, by analyzing the spatiotemporal dynamics of thousands of cells, we observed that ommatidial cells are carried along inside the MF, like surfers catching a wave, before exiting on the posterior side of the MF as rosettes. We are currently working to understand how spatiotemporal patterns of mechanical stress might regulate the emergence of stereotyped cellular geometry and, additionally, how cellular geometry feeds back onto the cell fate signaling contained therewithin.

Quantitative Analysis of Notch Signaling-Mediated Olfactory Neurogenesis
Jocelyn Garcia, Sriivatsan G. Rajan, Nathan Burg, Ankur Saxena

Poster presented by Jocelyn Garcia, University of Illinois at Chicago

The zebrafish olfactory epithelium (OE) is a complex tissue composed of two main types of olfactory sensory neurons (OSNs), microvillous (mOSNs) and ciliated (cOSNs). Previous data from our lab suggest that mOSNs and cOSNs are derived from two different stem cell populations, namely neural crest stem cells (NCCs) and placodal stem cells (PSCs), respectively. The highly conserved Notch signaling pathway is known to play a role in the proliferation and differentiation of cell lineages across multiple embryonic tissues. However, the molecular mechanisms through which Notch signaling regulates OSN differentiation remain unknown. We performed mRNA expression analysis and found that multiple Notch signaling components were expressed in the OE d¬¬¬uring olfactory neurogenesis. We next inhibited Notch signaling using both chemical and genetic methods and found statistically significant increases in the number of both OSN subtypes. Analysis of NCC differentiation in the OE revealed a higher rate of mOSN neurogenesis upon Notch signaling inhibition, which may explain the increase in mOSNs. Immunostaining for a PSC marker revealed a decrease in PSC numbers upon inhibition of Notch signaling. Additionally, mRNA expression for a neuronal progenitor marker in Notch signaling-inhibited embryos showed a decrease in the area of expression compared to control embryos, suggesting reduced numbers of neuronal progenitor cells. Taken together, these results suggest that the increase in mOSNs and cOSNs upon Notch signaling inhibition may be due to premature differentiation of their respective progenitor cells.

Notch Signaling Spatiotemporally Modulates Distinct Cellular Events During Olfactory Neurogenesis
Sriivatsan G. Rajan, Jocelyn Garcia, Nathan Burg, Kristin L. Gallik, Ankur Saxena

Poster presented by Sriivatsan Govinda Rajan, University of Illinois at Chicago

Defining the establishment of neuronal diversity at a system-wide level can provide fundamental insights into how a complex network of neurons is assembled. We use the zebrafish olfactory epithelium (OE) as a model system to delineate how distinct stem cell populations give rise to the two main types of olfactory sensory neurons (OSNs), ciliated and microvillous, during early development. Previous studies have reported the expression of Notch signaling receptors in the murine OE, but the downstream molecular mechanisms through which Notch signaling might regulate vertebrate OSN migration and differentiation have remained largely unexplored. We found that multiple components of the Notch signaling pathway exhibited dynamic expression patterns in the OE during early stages of olfactory neurogenesis. Furthermore, quantitative tracking of changes in Notch signaling activity of individual cells in our time-lapse imaging data during early olfactory neurogenesis revealed distinct clusters of cells with varying patterns of Notch signaling dynamics. Notch signaling knockdown using both chemical and genetic methods during specific time points yielded statistically significant increases in the numbers of both OSN subtypes, a decrease in the number of progenitor cells. Intriguingly, quantitative analysis of immunostaining for a progenitor marker across individual cells revealed that different subsets of progenitor cells exhibit discrete responses to Notch signaling perturbation. Taken together, our data suggest that dynamic spatiotemporal changes in Notch signaling play critical roles in regulating olfactory neurogenesis of

specific progenitor subpopulations. By better understanding Notch signaling-mediated olfactory development, we hope to discover conserved molecular mechanisms that regulate neuronal differentiation and organization in intricate organ systems.

Temporal precision of molecular events with regulation and feedback
Shivam Gupta, Sean Fancher, Erik Schild, Euclides E. Fernandes Póvoa, Hendrik C. Korswagen, Andrew Mugler

Poster presented by Shivam Gupta, Purdue University

Precise timing is crucial for many biological processes such as cell division, cell migration and embryonic development. However, these processes are governed by molecular events which are highly stochastic in nature. The regulatory mechanisms that cells use to suppress stochastic noise and achieve high timing precision are poorly understood. We investigate the regulatory strategies that maximally decrease timing noise. We confirm that autoregulatory feedback increases noise as found previously. Counterintuitively, we find that in the presence of regulation by a second species, autoregulatory feedback decreases noise. To explain this finding, we develop a method to calculate the optimal regulation function that minimizes the timing noise. The method reveals that the combination of feedback and regulation minimizes noise by maximizing the number of molecular events that must happen in sequence before a threshold is crossed. We compute the optimal timing precision for all two-node networks with regulation and feedback, derive a generic lower bound on timing noise, and test our results experimentally in the context of neuroblast migration during Caenorhabditis elegans development.

Physics of flow-sensing by self-communicating cancer cells
Nicholas Hilgert, Sean Fancher, Michael Vennettilli, Andrew Mugler

Poster presented by Nicholas Hilgert, Purdue University
Finalist Prize for Undergraduate Research in Quantitative Biology

The majority of cancer deaths occur when tumor cells metastasize, or spread throughout the body. Many cancer cells achieve metastasis by detecting the direction of flow in the lymphatic system. A prevailing hypothesis for this mechanism is that these cells self-communicate: they symmetrically emit molecules and asymmetrically re-absorb them after the molecules are displaced by the flow. This hypothesis is supported by experimental evidence [1], shown in Fig. 1A, but a quantitative theory of this process has not yet been constructed. We build a mathematical model founded on the physics of low-Reynolds number hydrodynamics and the mathematics of diffusive processes that sets limits on cellular sensing capabilities, providing a framework for experiments that will illuminate how physics informs the development of cancer.

Characterizing cell-cycle control of pluripotency and differentiation using ribosome profiling
Matt Hope, Keren Li, Jiping Wang, Alec Wang

Poster presented by Matt Hope, Northwestern University

Cells undergoing development are characterized by an uncommitted cell fate and increased capacity for proliferation, while differentiation marks an acquisition of cellular identity that is coupled with diminished or abolished capacity to proliferate. Therefore, cells must carefully balance these two key events in order to make functional tissues, yet the molecular underpinnings of this regulation are unclear. In mouse embryonic stem cells (mESCs), distinct phases of the cell cycle favor maintenance of pluripotentcy or exit toward a differentiated state. Transcriptional changes across the rapid cell cycle in mESCs are subtle, therefore we hypothesize that translational regulation is an under-explored mechanism for molecularly linking the cell cycle to proper timing of cell fate transitions. Here we propose using the genome-wide ribosome profiling technique to probe translation across the cell-cycle in mESCs. By using a genetically encoded fluorescent reporter of cell-cycle state, we will uncover both transcriptional and translational regulation in mESCs in order to build a more complete picture of the balance between proliferation and differentiation.

Epigenetic control of pluripotency in blastula and neural crest stem cells.
Paul Huber, Anjali Rao, Carole LaBonne

Poster presented by Paul Huber, Northwestern University

Neural Crest Cells (NCCs) are a stem cell population unique to vertebrate embryos. NCCs arise in the ectoderm at the neural plate border, and retain broad multi-germ layer developmental potential through neurulation. Much remains to be learned about the genetic and epigenetic mechanisms that regulate the retention of pluripotency in NCCs at stages when neighboring cells are undergoing lineage restriction. Here we report that the activity of epigenetic erasers, histone deacetylases or HDACs, as well as epigenetic readers of the BET (Bromodomain and Extra Terminal) family is essential for both maintenance of pluripotency in naïve blastula cells, and the establishment of NCCs. Blastula cells from embryos treated with pharmacological inhibitors of HDACs or BET factors BRD2, BRD3, and BRD4 lose pluripotency, as evidenced by down-regulated expression of key components of the pluripotency GRN, and are unable to respond to lineage instructing cues. Similarly, treatment of embryos with these inhibitors leads to decreased expression of NC markers at neurula stages. Because the phenotypic consequences of inhibiting a reader and an eraser of histone acetylation were strikingly similar, we compared the effects of HDAC and BET inhibition on the transcriptome. Our data show that expression of only a subset of genes is significantly altered in response to both inhibitors. Moreover, while HDAC inhibition leads to precocious and aberrant up-regulation of genes that direct multiple lineage states, loss of BET activity predominantly results in down-regulation of targets. Together these findings advance our understanding of the epigenetic control of pluripotency and the formation of the vertebrate neural crest and the role of lysine acetylation in these processes.

Physical constraints on epistasis
Kabir Husain and Arvind Murugan

Poster presented by Kabir Husain, University of Chicago

Living systems evolve one mutation at a time, but a single mutation can alter the effect of subsequent mutations. The underlying mechanistic determinants of such epistasis are unclear. Here, we demonstrate that the physical dynamics of a biological system can generically and easily constrain epistasis. We analyze generic models of proteins and regulatory networks. We find that if the long-time physical dynamics of the protein or regulatory network is dominated by a slow, collective mode, the dimensionality of mutational effects is reduced. Consequently, epistatic coefficients for different combinations of mutations are no longer independent, even if individually strong. In this case, epistasis can be summarized as resulting from a global non-linearity applied to an underlying linear trait, i.e., global epistasis. This constraint, in turn, reduces the ruggedness of the sequence-to-function map. By providing a generic mechanistic origin for experimentally observed global epistasis, our work suggests that slow collective physical modes can make biological systems evolvable.

Zebrafish as a model for mutations in sarcomeric proteins linked with human early-onset atrial fibrillation

Xinghang Jiang, Joseph Lombardo, Fritz Navales, Brandon Chalazan, Aylin Ornelas-Loredo, Denise Mol, Zain Alzahrani, Dawood Darbar, Ankur Saxena

Poster presented by Xinghang Jiang, University of Illinois at Chicago

Mutations in cardiac ion channels, signaling molecules, and structural proteins have been linked with familial (early-onset) atrial fibrillation (AF). Myosin heavy chain 6 (MYH6) encodes the alpha heavy chain subunit of cardiac myosin and is primarily expressed in the atrium. Titin (TTN) encodes the largest sarcomeric protein that is essential for sarcomere assembly and restoration of normal length after contraction. Although we have identified mutations in MYH6 and TTN associated with early-onset AF, the underlying pathophysiology by which these variants cause arrhythmia remains unclear. The zebrafish is a robust model to study cardiac AF pathogenesis because of a similar heart rate to humans and the ability to survive up to 7 days post-fertilization

without a functional heart. We have used CRISPR-Cas9 to generate mutant zebrafish harboring knock-in MYH6 and TTN mutations in their zebrafish orthologs. F0 mutant zebrafish have demonstrated atrial enlargement, pericardial edema, and marked atrial regurgitant inflow. Our preliminary findings suggest that the engineered MYH6 and TTN mutations yield distinct pathophysiological phenotypes. Ongoing studies of F1 progeny will provide insights into the underlying mechanisms by which these variants lead to AF.

Pattern formation in a membrane-bulk model for intracellular polarization and oscillations
Bin Xu, Frederic Paquin-Lefebvre, Kelsey Dipietro, Alan Lindsay, Alexandra Jilkine

Poster presented by Alexandra Jilkine, University of Notre Dame

Reaction-diffusion systems have been widely used by mathematical biologists to study spatio-temporal phenomena in cell biology, such as cell polarization. Coupled bulk-surface models naturally include compartmentalization of cytoslic and membrane-bound polarity molecules. Here we study the distribution of the polarity protein Cdc42 in a mass-conserved membrane-bulk model, and explore the effects of diffusion and spatial dimensionality on the types of solutions that are observed. We first analyze a one-dimensional (1-D) model for oscillations of Cdc42 and its GEF in fission yeast. In 1-D, the model includes a partial differential equation for diffusion of inactive species in the bulk and a pair of ordinary differential equations for binding kinetics at the two ends of the cell. Using linear stability analysis and numerical bifurcation analysis, we find that oscillations emerge via Hopf bifurcations. A supercritical Hopf bifurcation occurs for high diffusion rates of Cdc42/GEF while a subcritical Hopf bifurcation happens for low diffusion rates. Increasing the time that the GEF spends on the membrane results in a bigger parameter space where pole-to-pole oscillations are possible. We then extend our analysis to a two-dimensional (2-D) model. In 2-D, the species can either diffuse in the cell interior with no reaction kinetics, or become bound to the cell membrane and undergo surface diffusion with reaction kinetics. We also consider a nonlocal reduction of the 2-D model with a well-mixed interior. We perform linear stability analysis for both the nonlocal PDE system on a circular geometry, and the full two dimensional membrane-bulk system on a disk. In all three variants we find that mass-conservation selects perturbations of spatial modes that simply redistribute mass. In 1-D, only anti-phase oscillations between the two ends of the cell can occur, and in-phase oscillations are excluded. In higher dimensions, no radially symmetric oscillations are observed in numerical simulations. Instead, we observe stationary Turing patterns, as well as traveling and standing waves. The two types of waves coexist near the stability boundaries, while away from them standing waves are only transient. Our work clarifies the effect of geometry and dimensionality on behaviors observed in mass-conserved cell polarity models.

Quantitative Analysis of Transcriptome Dynamics During Developmental State Transitions
Kristin Johnson, Simon Freedman, Madhav Mani, Carole LaBonne

Poster presented by Kristin Johnson, Northwestern University

How a single cell ultimately gives rise to a patterned, complex organism is one of the most fundamental questions in biology. Throughout the course of embryonic development, the potency of cells become increasingly restricted, progressing from a pluripotent state to different lineage restricted states. Although gene expression at both the pluripotent start point and lineage restricted end point have been characterized, we lack a quantitative view of cell state transitions during developmental decision making. The pluripotent cells of Xenopus blastula-stage embryos are an optimal system in which to study these state transitions. The decision-making process spans hours rather than days in these cells, allowing dynamics to be studied at higher temporal resolution. We have used transcriptomics to interrogate the process by which pluripotent cells transit to two different lineage restricted states, neural progenitors and epidermis. Our experimental pipeline quantifies changes in gene expression across six time points during the ~7 hours these cells take to achieve lineage restriction. Our data uncovers dynamic changes in signaling pathways and gene regulatory networks during these transitions. Interestingly, analysis of transcriptome and pathway dynamics indicates that the transition from pluripotency to a neural progenitor state is unidimensional, whereas the trajectory to achieving an epidermal state is more complex, providing independent support for, and novel insights into, the neural default model. These studies provide quantitative insights into the logic and dynamics of stem cell decision making.

The Ca2+ Dependent Elasticity of Tip Link Cadherins in Hearing: Interfacing MD Measured Forces with Classical Mechanics Models
Colin Klaus, Bowen Jia, Shuqi Wang, Marcos

Poster presented by Colin Klaus, The Mathematical Biosciences Institute

Hearing signal transduction is mediated at a molecular level by the tip link, a cadherin complex comprised of PCDH15 and CDH23 repeats. Sound enters the ear as pressure waves whose mechanical energy is conveyed as tension by the tip link to open mechanically gated ion channels. Structural integrity of the tip link under tension depends on its occupancy by Ca2+ ions at discrete sites along the protein. This has led to 60,000+ different Ca2+ configurations, too large an ensemble to be studied directly using the Michaelis-Menten based probability distribution determined by site binding affinities and Molecular Dynamics (MD) alone. To overcome this limitation we take a data-driven approach. We interface forces measured from MD simulations with classical mechanics (Principal of Virtual Work) models. MD measured forces are decomposed into a deterministic component of one repeat acting on another and a random component due to the water bath and thermostat. Technically this is accomplished by a numerical conditional expectation of one random variable with respect to another and a step-function-discretization of the distribution for water bath and thermostat forces. This is joint work with The Sotomayor Lab at The Ohio State University Biochemistry Department.

Sensitivity Analysis of a Homogenized Diffusion Model for Cone Visual Transduction
Colin Klaus, Giovanni Caruso, Vsevolod Gurevich, Heidi Hamm, Emmanuele DiBenedetto

Poster presented by Colin Klaus, The Mathematical Biosciences Institute

Mammals have two types of photoreceptors, rods and cones. While rods are exceptionally sensitive and mediate vision at very low illumination levels, cones operate in daylight and are responsible for the bulk of visual perception. Through the mathematical techniques of homogenization and concentrated capacity, a novel diffusion model for cone visual transduction is presented and sensitivity analysis of model parameters is performed. This work also adapts a recently published dim light model of cone transduction to the case of high intensity light. This study is an interdisciplinary undertaking by mathematicians and pharmacologists at The Ohio State University, CNR IT, and Vanderbilt University.

A New Statistical Method for Testing Differential Ribosome Footprint Using Ribo-seq Data
Keren Li, Matthew Hope, Frank Fineis, Xiaozhong Wang, Ji-Ping Wang

Poster presented by Keren Li, Northwestern University

Emerging data suggest that the pluripotency of stem cells is regulated at the level of translation. We employ the recently developed Ribo-profiling technique to investigate translation dynamics in embryonic stem cells. High-density RNase footprints of ribosome-protected fragments provide quantitative and dynamic information of protein translation along mRNA coding sequence. However, most of current Ribo-profiling data analyses focus on testing the occupancy of ribosomes using the total read counts per open-reading form (ORF), while the detailed footprint patterns are largely ignored. We propose a new statistical method for testing the difference of ribosome footprint patterns for each gene in different biological conditions. We assume that read counts at each given position of a transcript follow negative binomial distributions. If the read counts across samples follow the same pattern in the entire transcript region, the largest eigenvalue of data matrix is shown to represent the signal strength while the rest for noise. We derive a test based singular value decomposition and apply it to real data to show its effectiveness in identifying genes with differential ribosome dynamic patterns genome-wide.

Parallel Screening and Rapid Identification of Orthogonal N-glycosyltransferases for Glycoprotein Engineering
Liang Lin, Weston Kightlinger, Michael Jewet, Milan Mrksich

Poster presented by Liang Lin, Northwestern University

Glycan modification is the most complex post-translational modification and can largely improve therapeutic effects of the protein, such as receptor binding, stability and serum half-life. To study the synergistic effects of glycan at different sites or install different glycans at different sites for synergistic functions, it is urgent to discover orthogonal glycosyltransferases (GTs), N-glycosyltransferases (NGTs) as an example for installing N-glycans. So we developed a systematic method to screen out orthogonal NGT-substrate pairs. First, we discovered the substrate binding residues and make hundreds of mutants potentially with different substrate selectivity. Second, we screened these mutants with the whole substrate library. Third, we developed a rapid identification method in excel to discover the orthogonal NGT pairs, for di-NGTs, to tetra-NGTs in a group. Finally, an orthogonal tetra-NGT-peptide substrate pairs was used to present the flow path for engineering different tetra-glycans in the same protein, with four peptide tags incorporated at different sites and four NGTs sequentially glycosylating each site.

Growth Control of Drosophila Wings
Andrew Liu, Madhav Mani, Richard Carthew

Poster presented by 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 during most of larval development before the onset of wing morphogenesis during the pupal stage. 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. My first aim is to quantify the expression gradients of Ds and Fj. I will also seek to genetically manipulate the expression gradients of these molecules and assay the changes in growth. This work will provide a systems level understanding of how growth control is conferred to tissue-scale systems.

Developmental regulation of cell type-specific transcription by novel promoter elements
Dan Lu, Hosu Sin, Chenggang Lu, Margaret T. Fuller

Poster presented by Dan Lu, Stanford University

The cell-type specific transcriptional programs that drive differentiation of specialized cell types are key players in both development and tissue regeneration. In Drosophila, one of the most dramatic transcriptional changes occur with the transition from proliferating spermatogonia to differentiating spermatocytes during spermatogenesis, which involves a third of the entire testis transcriptome. Here we show that most of the transcripts turned on in differentiating spermatocytes are expressed from spermatocyte-specific promoters that lack all of the previously identified canonical core promoter elements except for the Inr. Opening of these promoters from their closed state in precursor cells requires function of the spermatocyte specific tMAC complex, recruited locally to the promoters, resulting in a stretch of open chromatin of ~100bp in which transcription initiates. Within this open chromatin region, sequence motifs at or downstream of transcription start sites help promote efficient transcription at specific positions. Lastly, we provide evidence that a small group of extremely highly expressed promoters tend to associate with having several of these motifs at the optimal positions. Together, our results reveal how promoter proximal sequence elements and protein complexes that interact with them establish a robust, cell type specific transcription program for terminal differentiation.

Evaluating simulations of protein dynamics using novel, high-resolution data
Lauren McGough, Justin Kim, Eugene Klyshko, Sarah Rauscher, Rama Ranganathan

Poster presented by Lauren McGough, University of Chicago

Proteins are evolved molecular machines which carry out the chemical and mechanical processes required for life. Fundamentally, their functioning depends on motions of atoms, both local and collective, that span a wide range of length- and time-scales, making protein dynamics a challenge for experiment and simulation alike. Novel high-resolution time-resolved X-ray crystallography data have opened up a unique opportunity to evaluate the consistency between simulation and experiment. By simulating the experiment using different models and approximations, we are determining the conditions under which molecular dynamics predictions of protein dynamics agree with the data, thus providing a detailed test of physics-based simulations' capabilities and limitations.

Spatiotemporal control of cooperative behavior in yeast communities: Uncovering the role of interaction dynamics in determining community structure
Neydis Moreno Morales, and Megan McClean

Poster presented by Neydis Moreno Morales, University of Wisconsin-Madison

Interactions between microbes can be passive or active, and act over a range of length scales including local contact-dependent interactions and longer-scale diffusion-based interactions. The interactions can guide the development of complex communities. Community structure can depend on the cells present, their role in the community, their effects on fitness and their abundance in the population. Understanding the rules that govern the structure of microbial communities is a pressing issue to better understand microbe's role in the human microbiome and to develop the therapeutic potential of engineered consortia. I hypothesize that intermicrobe interactions are dynamic in time and space, and this regulation is essential for determining community structure. Determination of design rules requires the ability to manipulate both the interactions as well as the arrangement of species within the community. The work here focuses on the well-studied model of microbial cooperation; the sucrose utilization mechanism in the yeast, Saccharomyces cerevisiae. This system will be used to investigate the following aims: (1) Measure native dynamics of cooperative interactions in a yeast public goods system to determine the effect that environmental context has on determining community structure; and (2) Control the distribution of the public good to determine whether spatiotemporal factors are important for determining community structure.

Sub-cellular signaling events ranking for efficient drug design
Zeynab Mousavikhamene, Neda Bagheri

Poster presented by Zeynab Mousavikhamene, Northwestern University

Cell signaling incorporates multiple chains of events each of which play role in the development and progression of cancer. Inability to predict and control tissue level responses to tumor treatment from cellular and subcellular rules is one main obstacle of treatment failure for different types of cancers which causes alarming mortality rate. This problem motivates the development of a computational model that can be interrogated to uncover the cascading effect of drug modalities. In general, inhibiting one pathway will not necessarily halt cancer development and tumor can survive because of the complex nature of cellular processes. Thus, there exists a need to view the tumor holistically—to integrate our collected knowledge of individual tumor growth processes into a complex, multi-step, intertwined system where multiple interacting signaling processes play a crucial role on the final stage of tumor. The goal of this project is to holistically and systematically analyze in silico tumors in the context of a clinically relevant environment to identify areas of the signaling network to target in order to prevent tumor growth in vivo. My work will answer fundamental biological questions about the impact of changing subcellular mechanisms on population-level emergent behavior, and these answers will have implications in cancer drug-design. Ideally, my work will complement that of existing experimental work to guide clinicians and researchers toward new, effective treatment strategies.

Characteristics of microtubule steady states and their influence on the interpretation of experiments
Kristopher S. Murray, Ava J. Mauro, Holly V. Goodson

Poster presented by Kristopher Murray, University of Notre Dame

When studying Microtubule (MT) dynamics or regulation by MT binding proteins (MBPs), experiments are commonly performed at steady state. However, it is important to clarify what this means, as systems of microtubules undergoing dynamic instability can transiently pass through multiple steady states depending on the chosen experimental conditions. Experiments should be designed to study MTs in the appropriate steady state(s) to properly address the experimenter's hypothesis and minimize the potential for unclear results due to transient behaviors. We have used a simplified MT simulation in which MTs polymerize from stable seeds to identify the various MT steady states available under different, common experimental conditions. We are also examining the approximate timescales necessary to achieve them at different free tubulin subunit concentrations. In systems with constant total tubulin (competing, similar to polymerization in a closed test tube), MTs can reach two distinct steady states: first a polymer mass steady state and, much later, a length distribution steady state. In systems with constant free, soluble tubulin (noncompeting, similar to an open flow cell), the steady states depend on whether the free tubulin concentration is above or below a critical concentration for population growth. At [free tubulin] above this critical concentration, a system of MTs will reach a net growth steady state if given sufficient time, rather than steady states of polymer mass and length distributions. In addition to these steady states, any system of tubulin polymerizing under conditions of constant free tubulin will achieve a GTP cap length steady state. To illustrate the significance of these steady states for interpreting experiments, we have modified one of the kinetic parameters in the simulation, specifically the rate at which GTP subunits attach to depolymerizing GDP tips, to mimic the effect of a rescue-promoting MBP. These results show that the apparent effects of MBPs can depend on which steady state(s) the system is in when measurements are performed.

TimeTrial: An Interactive Application for Optimizing the Design and Analysis of Transcriptomic Times-Series Data in Circadian Biology Research
Elan Ness-Cohn, Marta Iwanaszko, William Kath, Ravi Allada, and Rosemary Braun

Poster presented by Elan Ness-Cohn, Northwestern University

The circadian clock, characterized by ~24 hour periodic patterns, drives the oscillatory dynamics of ~40% of genes across all tissues. While studies elucidating circadian phenomena were originally limited to western-blotting of core clock genes; in the era of high-throughout transcriptiomic analysis, cycling detection methods have been developed to analyze time-series gene expression data and identify genes oscillating with a circadian period. As experimental procedures become more complex with researchers preforming perturbation studies to elucidate mechanisms controlling the circadian clock, tools to assess and develop experimental protocols and select detection methods that optimize cycle detection is required for progressing the field of circadian biology. Here we benchmark four major cycle detection methods using synthetic and real datasets varying in sampling schemes, number of replicates, and biological noise. Our results highlight that no method outperforms all others across datasets, and that the optimal choice of method depends on the noise level, number of missing data-points, number of replicates, sampling scheme used, and shape of the waveform of interest. We further present a framework for improving computational methods and experimental design, while simultaneously attempting to minimizing experimental cost. We have developed an interactive application to help users select the best method and sampling scheme for their experiment, as well as allow researchers to probe datasets for cycling genes of interest. Our freely available data and application will aid in the enhancement of rigor and reproducibility in future time-series experiments and improved tools to analyze cycling genes in increasingly large and complex time-series datasets.

Quantitative High-Throughput Measurements of Growth in C. elegans
Joy Nyaanga, Gaotian Zhang, Niall Mangan, Erik Andersen

Poster presented by Joy Nyaanga, Northwestern University

Organismal growth and development are driven by several factors. In organisms, growth is regulated on a genetic level, as changes in gene expression patterns and signaling dictate much of development. However, environmental conditions (e.g. nutrients and temperature) also exhibit strong impact on developmental growth rates. As animals consume and breakdown food, temperature dictates the metabolic rate at which these diverse nutrients are processed. In this way, environmental factors are directly relayed through metabolism to shape overall growth. For a comprehensive understanding of organismal growth and development, the link among genetics, environment and metabolic regulation must be considered. We investigated this connection in the roundworm Caenorhabditis elegans using a high-throughput phenotyping platform. This method allows for dense temporal sampling at high replication. We began by analyzing growth from hatching to adult of six independent populations of 100,000 nematodes fed Escherichia coli strain HT115 at 20°C. The resulting growth curves exhibited piecewise linear behavior, revealing distinct points where growth rate changes. This suggests that systemic adjustments are occurring in the nematode at these time-points, thereby modifying overall organism growth. Further analysis of C. elegans exposed to varying temperature and food source will identify whether these growth patterns are altered by varying environmental input. Furthermore, integration of gene expression and metabolic data will provide insight to how genetics and environment work together to influence nutrient use and biomass production in the developing organism.

Pattern recognition through molecular self-assembly
Jackson O'Brien, Constantine Evans, Erik Winfree, Arvind Murugan

Poster presented by Jackson O'Brien, University of Chicago

The functional role of many weak promiscuous interactions among molecules in biology is not clear and is often assumed to be deleterious. Here, we exploit promiscuous interactions to engineer an experimental system of 917 single-stranded DNA molecules capable of associative pattern recognition on the high dimensional concentration patterns of these molecular assembly components. Such pattern recognition is achieved by exploiting a process of competitive nucleation between different polymorphic DNA structures that are predominantly made of the same molecules but co-localized in different combinations. We test the system with numerous concentration patterns and confirmed nucleation-based pattern recognition through Atomic Force Microscopy (AFM) and fluorescence measurements. We discuss how this system, in conjunction with additional enzymatic components (e.g. DNA ligase), can potentially learn the promiscuous interactions needed to perform unsupervised clustering of concentration patterns.

Using models to inform the effectiveness of antimalarial drugs for seasonal malaria chemoprevention
Kamaldeen Okuneye, Jaline Gerardin

Poster presented by Kamaldeen Okuneye, Northwestern University

Seasonal malaria chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) is recommended as an additional intervention against Plasmodium falciparum malaria for children under the age of 5 years in the Sahel sub-region of Africa, where over 60% of clinical malaria cases occur during the 3 – 4-month rainy season and about 90% of the mortality and morbidity occurs among children under the age of 5 years. The aim of SMC is to prevent illness by maintaining therapeutic antimalarial drug concentrations in the blood throughout the malaria period. Randomized control trials have shown SMC to be effective, safe, relatively cheap and typically reduces clinical malaria episodes by approximately 75%. This study aims to highlight the determinants of the therapeutic effectiveness of SP-AQ drugs by quantifying the curative and prophylactic properties of the antimalarial drugs.

Pattern and Stripe Formation in Zebrafish
Addie Harrison, Gisela Hoxha, Gil Parnon, Madison Russell, Bjorn Sandstede, Berke Turkay, Alexandria Volkening

Poster presented by Gil Parnon, Portland State University
Finalist Prize for Undergraduate Research in Quantitative Biology

Zebrafish (Danio Rerio) are fish that live in freshwater and have black stripes and yellow interstripes. The stripes are made of a combination of melanophores, iridophores, and xanthophores, the latter two have both loose and dense forms. This REU project built on the work of a paper by Sandstede and Volkening to extend and develop their model to be both more accurate and applicable onto the fins of the zebrafish as well as implementing skin growth and bones onto a new domain. Previous models only took melanophores and xanthophores into account. Over the course of this project we further developed a 5-cell model in order to advance the biological understanding of how autonomous stripe formation works on zebrafish. We used a series of differential equations to describe interactions between cells, bones, and the edges of the simulated fish body. This work incorporated mutated variations of Danio Rerio which were missing certain pigment cell types in order to better replicate a realistic model. By accurately being able to generate mutations as well as the unmutated fish we were able to check for accuracy of the cell-to-cell interactions. We developed a methodology for generating and working with boundaries of different shapes in order to accommodate different fins as well as more realistic bodies, and worked towards biological realism in our model.

A new biomechanical mathematical model to predict force effects on actin dynamics during clathrin-mediated endocytosis
Ruchira Ray, Julien Berro

Poster presented by Ruchira Ray, Yale University
Finalist Prize for Undergraduate Research in Quantitative Biology

Background: During clathrin-mediated endocytosis, a flat membrane invaginates to form a round vesicle. In yeast, actin filaments assemble into a network that generates the force necessary to deform the membrane. Mechanical models have described how endocytic proteins generate force, while biochemical models have described how their composition changes over time. However, the interactions between the mechanical and chemical aspects remain unclear. Objective: This study proposes a new biomechanical approach to model how the actin filament network composition responds to different load forces in yeast. Methods: The biochemical component of the model was a system of ordinary differential equations representing the key reactions involved in actin assembly and disassembly, used in a previous study and validated by in vivo yeast data. We added mechanical relationships to the reactions using alternative hypotheses of how the load force changes over time. The Arrhenius/Bell equation was used to specify an inverse relationship between load force and the actin polymerization rate constant. We assessed two different hypotheses. In the “constant load model”, the load force remains constant throughout endocytosis (figure 1A, left). In the “variable load model”, the polymerizing actin filaments increase the load force, creating a negative feedback loop: as more filaments polymerize, the load force increases, inhibiting polymerization (figure 1B, left). We proposed the “variable load model” because actin filaments generate and experience force during endocytosis, so a force-feedback could regulate actin assembly. We simulated the time evolution of the components of the actin filament network including F-actin and capped filament ends (capping protein). We assessed the performance of the models using two criteria: the number of peaks and the peak value of the time evolution of the copy number of each protein.

Dorsal/NF-κB exhibits a dorsal-to-ventral mobility gradient in the Drosophila embryo
Hadel Al Asafen, Natalie M. Clarkb, Thomas Jacobsen, Rosangela Sozzani, and Gregory T. Reeves

Poster presented by Gregory Reeves, North Carolina State University

Morphogen-mediated patterning is a highly dynamic developmental process. To obtain an accurate understanding of morphogen gradients, biophysical parameters such as protein diffusivities must be quantified in vivo. The dorsal-ventral (DV) patterning of early Drosophila embryos by the NF-κB homolog Dorsal (Dl) is an excellent system for understanding morphogen gradient formation. Dl gradient formation is controlled by the inhibitor Cactus/IκB (Cact), which regulates the nuclear import and diffusion of Dl protein. However, quantitative measurements of spatiotemporal Dl movement are currently lacking. Here, we use scanning fluorescence correlation spectroscopy to quantify the mobility of Dl. We find that the diffusivity of Dl varies along the DV axis, with lowest diffusivities on the ventral side, and the DV asymmetry in diffusivity is exclusive to the nuclei. Moreover, we also observe that nuclear export rates are lower in the ventral and lateral regions of the embryo. Both cross correlation spectroscopy measurements and a computational model of Dl/DNA binding suggest that DNA binding of Dl, which is more prevalent on the ventral side of the embryo, is correlated to a lower diffusivity and nuclear export rate. We propose that the variation in Dl/DNA binding along the DV axis is dependent on Cact binding Dl, which prevents Dl from binding DNA in dorsal and lateral regions of the embryo. Thus, our results highlight the complexity of morphogen gradient dynamics and the need for quantitative measurements of biophysical interactions in such systems.

Acute Rho1 activation reveals that ventral epithelial cells of the Drosophila embryo are specifically predisposed for coordinated anisotropic constriction during gastrulation
Ashley Rich, Richard Fehon, Michael Glotzer

Poster presented by Ashley Rich, University of Chicago

Many morphogenetic events, including convergent extension and tube formation, require modulation of the actomyosin cytoskeleton. Ventral furrow formation in Drosophila embryos is one such morphogenetic event; it results when extracellular signals activate two transcription factors, Snail and Twist, in a subset of ventral epithelial cells. These regulators then drive the expression of multiple proteins which ultimately induce Rho1 activation, apical constriction, and invagination of cells into the embryo. These apical constrictions are anisotropic and appear coordinated, but the basis for this anisotropy and coordination is unknown.

To address this and related questions, we utilized optogenetics to control Rho1 activity in the embryo. Acute Rho1 activation at the onset of gastrulation induces ectopic invaginations in both the dorsal and ventral embryonic epithelium. Rho1 activation induces apical constriction in both dorsal and ventral cells, but ventral cell constriction is stronger and more anisotropic. Strikingly, ectopic Rho1 activation induces non-cell autonomous deformations outside the activation zone only in the ventral epithelium. Thus, we demonstrate that ventral cells are specifically predisposed to respond to Rho1 activation with anisotropic and coordinated deformations.

To identify the factors required for ventral cell specific behavior, we analyzed acute Rho1 activation in embryos deficient in factors required for ventral furrowing. Ventral cells depleted of RhoGEF2 exhibit anisotropic apical constriction, suggesting a molecular specialization of ventral cells beyond their ability to activate high levels of Rho1. Ventral cells lacking Twist exhibit weaker and less anisotropic apical constrictions, indicating a previously unknown role of Twist. Unlike wildtype or twist embryos, Rho1-induced deformations persist in RhoGEF2-depleted embryos.

Collectively, our results demonstrate that while Rho1 is sufficient to initiate invagination throughout the embryonic epidermis, the individual cell shape changes accompanying these invaginations differ between dorsal and ventral cells. Additionally, ventral cells specifically can propagate the response to Rho1 activation outside of the zone of optogenetic activation. Experiments are in progress to identify the factors required for these ventral specific behaviors.

Modeling DNA Replication using the sine-Gordon Equation
Susan Rogowski, Sarah Raynor

Poster presented by Susan Rogowski, Florida State University

DNA replication begins when locally unzipped regions of several broken hydrogen bonds form which cause a partial unwinding of the double helix. These regions are often referred to as bubbles and their formation can be modeled using a chain of coupled pendula. The angular oscillations that occur are usually modeled using the sine-Gordon equation. In this model, the discrete analog of the sine-Gordon equation is derived. Instead of taking the continuous limit, the space step between pendula remains nonzero and the forces on each pendulum are considered. Additionally, the randomization of nitrogen bases, Adenine, Thymine, Guanine, and Cytosine, within the chain of pendula, is modeled by randomly selecting the corresponding coefficients for each base. The system of differential equations is solved and plotted using Verlet integration in MATLAB.

Effects of cell-cell adhesion on collective migration of multicellular clusters
Ushasi Roy, Andrew Mugler

Poster presented by Ushasi Roy, Purdue University

Collections of cells exhibit directional coherent migration during embryonic development, metastatic cancer and wound healing. Often, the position of a particular cell within a cluster is not fixed while executing such motion. As a result, bigger clusters split, smaller sub-clusters collide and reassemble and gaps continually emerge. This leads to the formation of protrusions by some inner cells which eventually act as “leaders” along with the cells at the leading edge, pulling the cluster towards the favorable direction. Studies have shown that these phenomena are often observed in large populations like neural crest cells. We hypothesize that the cells may have an optimal adhesion among themselves, rather than very strong or weak, to favor the formation of gaps and achieve an effective faster migration. We develop a computational model of a two dimensional cell sheet based on the lattice gas model of statistical physics, from which we compute statistical averages of collective properties such as cluster velocity. The numerical results agree with our analytical calculations. For capturing the dynamical behavior, we also study this phenomenon by employing the cellular Potts model based on contact inhibition of locomotion.

Tuning environmental timescales to evolve and maintain generalists
Vedant Sachdeva, Kabir Husain, Jiming Sheng, Shenshen Wang, Arvind Murugan

Poster presented by Vedant Sachdeva, University of Chicago

Natural environments can present diverse challenges, but some genotypes remain fit across many environments. Such `generalists' can be hard to evolve, out-competed by specialists fitter in any particular environment. Here, inspired by the search for broadly-neutralising antibodies during B-cell affinity maturation, we demonstrate that environmental changes on an intermediate timescale can reliably evolve generalists, even when faster or slower environmental changes are unable to do so. We find that changing environments on timescales comparable to evolutionary transients in a population enhances the rate of evolving generalists from specialists, without enhancing the reverse process. The yield of generalists is further increased in more complex dynamic environments, such as a `chirp' of increasing frequency. Our work offers design principles for how non-equilibrium fitness `seascapes' can dynamically funnel populations to genotypes unobtainable in static environments.

Antagonism in multiple-cue chemotaxis in breast cancer cells
Soutick Saha, Hye-ran Moon, Bumsoo Han and Andrew Mugler

Poster presented by Soutick Saha, Purdue University

Chemotaxis is defined as biased cell motion towards an external chemical gradient. It is a pivotal step in cancer metastasis where cancer cells move towards chemical cues and spread to different parts of the body. We used triple-negative breast cancer cells to study chemotaxis towards cues formed by multiple growth factors and found, surprisingly, that the bias in the movement was less pronounced when we combine two attractant gradients compared to when we have individual gradients. We study this antagonism using Gillespie simulations and a simple analytic model.

Tractions control cell shape and rearrangements in collective cell migration
Aashrith Saraswathibhatla, Jacob Notbohm

Poster presented by Aashrith Saraswathibhatla, University of Wisconsin-Madison

Key to collective cell migration is the ability of cells to rearrange their position with respect to their neighbors. Recent theory and experiments demonstrated that cellular rearrangements are facilitated by cell shape, with cells having more elongated shapes and greater perimeters more easily sliding past their neighbors within the cell layer. Though it is thought that cell perimeter is controlled primarily by cortical tension and adhesion at each cell's periphery, experimental testing of this hypothesis has produced conflicting results. Here we studied collective migration in an epithelial monolayer by measuring forces, cell perimeters, and motion, and found all three to decrease with either increased cell density or inhibition of cell contraction. In contrast to previous understanding, the data suggest that cell shape and rearrangements are controlled not by cortical tension or adhesion at the cell periphery but rather by the stress fibers that produce tractions at the cell-substrate interface. This finding is confirmed by an experiment showing that increasing tractions reverses the effect of density on cell shape and rearrangements. Our study therefore moves focus away from the cell periphery and establishes cell-substrate traction as the physical factor controlling shape and motion in collective cell migration.

Modeling Blood Flow Regulation and Tissue Oxygenation in the Retina
Hannah Scanlon, Mandy Abernathy, Brendan Fry, Julia Arciero

Poster presented by Hannah Scanlon, Wake Forest University
Finalist Prize for Undergraduate Research in Quantitative Biology

Glaucoma is a serious ocular disease characterized by damage to retinal ganglion cells that results in irreversible vision loss. While thought primarily to be a disease associated with high intraocular pressure, clinical evidence shows that almost one third of glaucoma patients do not exhibit elevated pressure. Impaired tissue perfusion, blood flow, and oxygenation of retinal tissue have been identified as other important factors that may contribute to retinal ganglion cell death. Theoretical modeling provides a useful tool for predicting the impact of several hemodynamic factors on blood flow regulation and retinal oxygenation. In this study, two mathematical models (compartmental and heterogeneous) are combined into a hybrid model to describe tissue oxygenation in a human retina. A compartment model that represents blood vessels as resistors is adapted to include a wall-derived metabolic signal. This signal is calculated from the partial pressure of oxygen in the blood and assumed to be conducted upstream through the network. The model is used to predict changes in retinal tissue perfusion as tissue oxygen demand is increased or as incoming arterial pressure is varied. A heterogeneous model of the human retinal arterioles is adapted from a mouse model. The heterogeneous model is connected to a compartmental model of the capillaries and venules to create a hybrid model description of the retinal microcirculation. Flow, pressure, and partial pressure of oxygen from the heterogeneous model serve as inputs to the compartmental model, and then the conducted metabolic signal is calculated in the capillaries and venules and relayed back to the heterogeneous arterial network. This hybrid model preserves important spatial information from the arteriolar network while accounting for realistic conducted metabolic responses generated downstream. Ultimately, this model provides an important approach for predicting retinal blood and tissue oxygenation within a realistic human retinal network and understanding the role of these factors in glaucoma.

Crosstalk Between MAPK Networks in Closely Related Strains of Yeast
Taylor D. Scott, Ping Xu, Megan N. McClean

Poster presented by Taylor Scott, University of Wisconsin-Madison

In budding yeast, Saccharomyces cerevisiae, three mitogen-activated protein kinase (MAPK) pathways (the high osmolarity glycerol (HOG), mating, and filamentous growth (FG) pathways) govern the responses to osmotic stress, pheromone, and nutrient limitation respectively. Signaling through each of these pathways passes through shared kinases (Ste20p, Ste11p) yet despite this shared reaction, there is little-to-no cross-activation of the mating and FG pathways in response to osmotic stress. We use single cell experiments with fluorescent reporters to dissect how network dynamics and structure control crosstalk between the HOG and mating/FG MAP kinase pathways in two closely related strains of yeast. We find that the level of crosstalk is strain-dependent, with one strain showing no crosstalk and a second strain showing significant leakage into the mating and FG pathways after osmotic shock. We find that this crosstalk is dose-dependent with the highest levels of crosstalk occurring at moderate dosages of sorbitol. We use deletion strains to probe the effect of osmotic stress on the mating and FG pathways together and separately. Future work will explore how the differences between the two strains leads to differential levels of crosstalk at the same level of osmotic stress.

Inspection of high-resolution microtubule length history data using a novel dynamic instability analysis tool reveals additional statistically significant phase
Jared P. Scripture, Shant Mahserejian, Ava J. Mauro, Elizabeth J. Lawrence, Erin Jonasson, Kristopher M. Murray, Jun Li, Melissa Gardner, Mark S. Alber, Marija Zanic, and Holly V. Goodson

Poster presented by Jared Scripture, University of Notre Dame

Microtubules (MT) are vital components of the eukaryotic cytoskeleton that exhibit periods of slow growth stochastically interrupted by rapid depolymerizations (dynamic instability, DI). Study of MTs is dependent on the ability to quantify and describe this DI behavior accurately, as effects of microtubule binding proteins (MTBPs) and other regulatory factors are often determined by comparing DI metrics. MTs have long been analyzed as two-state polymers (i.e., MTs are either growing or shortening) with instantaneous transitions. However, recent advances in microscopy have allowed for the capturing of data with higher spatiotemporal resolution where qualitative observations point to ambiguous behaviors that are not clearly identifiable as growth or shortening. This suggests a need for a more flexible method, not constrained by the two-state assumption, that is capable of considering the entirety of MT behavior. With this in mind, we developed STADIA, an unbiased, automated method rooted in machine learning to more accurately identify, classify, and analyze macro-level phases exhibited by MTs. Applying this novel method to both in silico and in vitro MT length history data revealed multiple statistically significant behaviors within growth and shortening as well as a third, predominant phase, which we termed “stutters”, where relatively little length change occurs. Stutter phases were found primarily preceding catastrophes, suggesting that they are an important step mechanistically in transitioning from growth to shortening. Additionally, MTBP CLASP2γ was found to cause significant changes in phase metrics for in vitro MTs. We conclude that this new approach not only yields more complete, accurate measurements compared to classic methods, but also allows for discovery of important phases that are crucial to understanding MT behavior. Furthermore, the identification of stutters by this tool provides a promising target for mechanistic studies on phase transitions.

Exclusion of Metastatic Neuroblastoma via Microenvironment-Induced, ITSN1-Dependent Differentiation In Vivo

Randall Treffy, Sriivatsan Govinda Rajan, Lynne Nacke, Usama Malkana, Dani Bergey, Dianicha Santana, John O'Bryan, Ankur Saxena

Poster presented by Randall Treffy, University of Illinois at Chicago

Neuroblastoma (NB) is the most common cancer in the first year of life and is derived from neural crest cells (NCCs), highly migratory, multipotent stem cells that give rise to many cell types including neurons, glia, and melanocytes. Despite NCCs migrating and differentiating throughout the embryo, including making major contributions to the developing head, NB is found almost exclusively within the abdomen and thorax for reasons that remain unclear. Intriguingly, NB in young patients exhibits a high rate of spontaneous resolution, with malignant cells often undergoing apoptosis or differentiating into non-malignant neurons. We hypothesized that spontaneous resolution might play a role in NB's trunk-biased localization in patients and devised an in vivo experimental system to test this idea. First, we injected human NB cells into zebrafish embryos alongside endogenous NCCs at a specific location and time when some NCCs migrate towards the head and others migrate away from it. We tracked both cell types over time in vivo with high-resolution microscopy and found that NB cells migrated along endogenous NC pathways while non-cancerous cells did not. When we injected a NB cell line, SK-N-AS, that is incapable of differentiating into neurons in vitro, we found that, as expected, SK-N-AS cells migrating away from the head did not differentiate in vivo as well. Strikingly, however, most SK-N-AS cells that migrated into the developing head differentiated into neurons. Next, we treated SK-N-AS cells prior to injection with retinoic acid (RA), a commonly used but poorly understood chemotherapeutic agent for NB patients that cannot induce SK-N-AS differentiation in vitro. Many RA-treated, injected SK-N-AS cells, including those in locations previously unable to induce differentiation in vivo, rapidly became neurons. We also explored the role of intersectin-1 (ITSN1), a scaffolding protein with known tumorigenic properties, in our microenvironment.

We injected ITSN1-deficient SK-N-AS cells and found a stark decrease in their ability to differentiate into neurons. To test the possibility of crosstalk between RA signaling and ITSN1, we treated ITSN1-deficient NB cells with RA pre-injection, which yielded a partial rescue and/or override of ITSN1-silencing and restored neuronal differentiation in vivo in a subset of injected cells. Taken together, our findings suggest that 1) environmental cues from the developing nervous system actively exclude NB cells from the head by inducing them to differentiate into neurons that are no longer capable of metastasis, whereas the trunk may provide a more permissive environment for NB malignancy; 2) RA may function effectively as a NB treatment due to its rapid promotion of neurogenesis; 3) ITSN1 may play a critical role in NB malignancy. Our data provide a novel explanation for NB's trunk-biased anatomical localization in young patients and highlight the likely importance of microenvironment-induced differentiation in promoting NB's spontaneous resolution.

Multistationarity in the multi-site sequential phosphorylation mechanism is determined by catalytic constants

Maya Mincheva and Thomas Ventimiglia.

Poster presented by Thomas Ventimiglia, Northern Illinois University

A sequential distributive mechanism for n-site phosphorylation and dephosphorylation of proteins is analyzed as a system of ordinary differential equations. It is shown for the n=2, 3, and 4 cases that multistationarity is possible if the catalytic constants satisfy a simple inequality. We conjecture that similar results hold for all positive integers n.

Topological techniques for quantifying pattern variability in zebrafish
Melissa McGuirl (Brown University) and Bjorn Sandstede (Brown University)

Poster presented by Alexandria Volkening, Northwestern University

Wild-type zebrafish feature black and yellow stripes across their body and fins, but mutants display a range of altered patterns, including spots and labyrinth curves. All these patterns form due to the interactions of pigment cells, which reliably self-organize to create robust stripes despite their stochastic environment. With the goal of helping link genes to their functional impact on cell behavior, agent-based models have previously been developed to simulate wild-type and mutant pattern formation on zebrafish. Pattern features are generally analyzed visually, however, and this limits the scale of model parameter screens to identify cell interactions that may be associated with mutants. Moreover, within a single zebrafish mutation, there is a lot of pattern variability in vivo, and this makes it challenging to objectively identify the distinguishing features of a given pattern that a model is trying to reproduce and then judge model performance. To help address these challenges, we develop a set of tools, based in topological data analysis and interpretable machine learning, for automatically quantifying pattern features at large-scale. By analyzing thousands of simulated patterns, we suggest distinguishing features of different zebrafish mutants, determine the impact of different model parameters without the need for visual inspection, and predict how variable wild-type and mutant patterns are in vivo.

Measuring Transcription at a Single Gene Copy Reveals Hidden Drivers of Bacterial Individuality
Mengyu Wang*, Jing Zhang*, Heng Xu, Ido Golding

Poster presented by Mengyu Wang, University of Illinois at Urbana-Champaign

Single-cell measurements of mRNA copy-number inform our understanding of stochastic gene expression, but these measurements coarse-grain over the individual copies of the gene, where transcription and its regulation stochastically take place. Here we combine single-molecule quantification of mRNA and gene loci to measure the transcriptional activity of an endogenous gene in individual Escherichia coli bacteria. Interpreted using a theoretical model for mRNA dynamics, the single-cell data allows us to obtain the probabilistic rates of promoter switching, transcription initiation and elongation, mRNA release and degradation. Unexpectedly, we find that gene activity can be strongly coupled to the transcriptional state of another copy of the same gene present in the cell, and to the event of gene replication during the bacterial cell cycle. These gene-copy and cell-cycle correlations demonstrate the limits of mapping whole-cell mRNA numbers to the underlying stochastic gene activity, and instead highlight the contribution of previously hidden variables to the observed population heterogeneity.

Quantitative Analysis of Four-Dimensional Cell Movements in vivo During Olfactory Neurogenesis
Vijay Warrier, Joseph Lombardo, Celine Cluzeau, Chen Bi-Chang, Dani Bergey, Eric Betzig, Ankur Saxena

Poster presented by Vijay Warrier, University of Illinois at Chicago

Olfactory neurogenesis involves the contributions of multiple stem cell populations (the neural crest and ectodermal placode) to form sensory neurons. To understand the relationships between these populations during development we need to characterize complex four-dimensional phenotypes. We used the embryonic zebrafish and lattice light-sheet microscopy to accurately track cranial neural crest cells (NCC) and placode cell (PLC) movements in vivo. Analyzing cell distance to anatomical landmarks revealed that PLCs and subsets of NCCs exhibit distinct motion. Correlation analysis suggests that cellular motion is noisy and tests for stationarity indicate changes in these noise profiles across time and cell type. Statistically fitted models provide evidence of dynamic stochastic equilibration of mean separation of displacements between and within subsets of NCCs and PLCs (these cells act as if they are 'elastically tethered'). Further evidence of this dynamical coordination is provided by changes in the deviation of displacements of the equilibria trajectories over time (synchronization). Our results reveal a highly dynamic interplay between different progenitor cell types during the formation of the olfactory system and demonstrate how unbiased quantitative and statistical methods can advance the characterization of complex developmental patterns.

Building a planar signaling system that directs actin protrusion and collective migration of epithelial cells.
Audrey M. Williams and Sally Horne-Badovinac

Poster presented by Audrey Williams, University of Chicago

Cells migrating as a collective need to ensure that their leading and trailing edges align with their neighbors, a form of planar cell polarity. We study how a novel planar cell polarity system in the Drosophila egg chamber epithelium coordinates one such collective migration. The follicle cells that make up the egg chamber epithelium undergo a highly coordinated collective migration along an underlying basement membrane extracellular matrix. The tissue lacks leader cells, and tissue-level polarity emerges from cell-cell interactions across the field. We show that Fat2, an atypical cadherin that localizes to the trailing edge, promotes lamellipodial protrusion by recruiting the Wave complex to clusters at the leading edge of the cell behind. Fat2 also recruits Lar and Sema-5c, two transmembrane signaling proteins, to these leading edge clusters to form a planar-polarized intercellular signaling complex. Acute disruption of Fat2 signaling with the calcium chelator EGTA causes dissociation of Lar, Sema-5c, and the Wave complex, and abruptly halts migration. Upon restoration of calcium, the Fat2 signaling complexes reassemble, the tissue selects a new planar-polarization vector, and migration resumes. With this manipulation, we can use live imaging to watch the entirety of tissue polarization and signaling complex assembly. Key next questions include 1) How does the Fat2 signaling complex organize asymmetrically across cell-cell junctions and 2) How does polarity information propagate across the cell field?

Cell cycle regulation, inter-organelle communication and organelle homeostasis as drivers of cell fate in budding yeast
N. Ezgi Wood, Piya Kositangool, Ashley Marchand, Hanaa Hariri, Mike Henne

Poster presented by N Ezgi Wood, University of Texas - Southwestern Medical Center

In the face of unpredictable nutrient fluctuations, budding yeast cells must adapt to transitions between nutrient rich and poor conditions so that they maintain viability and proliferative capacity during starvation, and resume growth following an influx of new nutrients. Such adaptations are achieved through coordinated remodeling of numerous cellular systems encompassing nutrient sensing, cell cycle, stress response, metabolic, and energy homeostasis pathways. Different cellular organelles must also collaborate, often by communicating via inter-organelle contacts, to execute adaptive metabolic responses [1]. Depending on the strength and type of nutrient deficiency, the adaptive starvation responses can result in diverse cell fates including quiescence, senescence, pseudohyphal growth, and meiosis [2]. Importantly, these different fates co-exist in a population of genetically identical cells, implying additional non-genetic factors also govern cell fate decision-making [3, 4]. Although yeast cell cultures have been extensively studied through nutrient transitions using bulk population-based methods, how this adaptation to nutrient fluctuations is coordinated at the single cell level, and how this response diverges among cells with different fates within a clonal population, remain unresolved questions.

To address these, we used the quiescence/senescence decision making in budding yeast as a model system. The cells were grown in a microfluidics chamber and were followed with live-cell time lapse imaging before, during, and after acute glucose starvation (AGS). Upon exposure to the rich medium, some cells resume mitotic cycles (quiescence), whereas some do not, although being metabolically active (senescence). We simultaneously tracked cell cycle, stress response, inter-organelle communication and metabolic markers, and showed that both subpopulations can sense the nutrient starvation and activate canonical stress response pathways. Yet, during the nutrient starvation the cell cycle regulation, inter-organelle communication and organelle homeostasis markers of the two subpopulations diverge. For instance, although quiescent cells translocate the cell cycle inhibitor Whi5 to nucleus and come to a G1-like state during AGS, senescent cells fail to achieve this translocation. In addition, during the AGS the senescent cells fail to enlarge their nuclear-vacuolar junctions and lose vacuolar homeostasis marked by their inability to merge endocytotic vesicles with the vacuole. We also followed Rim15, which is an effector kinase under multiple nutrient sensing pathways, and demonstrated that the Rim15 cellular levels before AGS correlates with the cell fate after AGS.

Overall, we show that cell fates are established during AGS before the return to the rich medium, i.e. before the networks that are responsible for the realization of the cell fate (to cycle or not) are activated. Our data supports the model where failure to achieve proper adaptation and losing organelle homeostasis prevents senescent

cells from resuming mitotic cycles upon glucose replenishment. The stochastic variations in the nutrient sensing pathway components before the AGS might be responsible for the differential adaptation during AGS.
Gouleme L, Jimenez L, Khemiri I, Sagot I. Mitochondria reorganization upon proliferation arrest predicts individual yeast cell fate.

Kinesin-directed secretion shapes the extracellular matrix
Allison L. Zajac, Adam J. Isabella, Kriza E. Sy, Sally Horne-Badovinac

Poster presented by Allison Zajac, University of Chicago

The sorting of newly synthesized proteins into apical or basolateral secretory pathways is an important step in maintaining epithelial cell polarity. Although the basolateral pathway encompasses many different cargos that ultimately populate distinct basal or lateral plasma membrane domains, little is known about the role of targeted secretion to subdomains within the larger basolateral plasma membrane. One developmentally important group of basolateral cargos is the components of the basement membrane (BM). The BM is a dense, sheet-like extracellular matrix that exclusively lines the basal surface of epithelial cells, where it provides an attachment site for cells, a reservoir of growth factors, and mechanical strength that can guide tissue morphogenesis. Since BM proteins are designed to form networks upon exposure to the extracellular environment, control over the site of their secretion may be particularly important. Working in the follicular epithelium of Drosophila, our lab previously identified the GTPase Rab10 as a key regulator of polarized BM secretion that directs BM secretion to the lateral plasma membrane. Here, we identify a kinesin-1 and kinesin-3 (Khc73)-based transport pathway that takes advantage of the microtubule (MT) organization in these cells to focus secretion of BM proteins on a basal subregion of the lateral plasma membrane. This is one of the first studies to demonstrate a role for MT motors in basolateral secretion and suggests further study of the MT motor-driven transport of different cargos in epithelial cells may shed new light on how epithelial cells maintain their distinct polarized domains.

Cell Division Rate Controls Cell Shape Remodeling in Epithelia
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Epithelia have distinct cellular architectures, which are established in development, re-established after wounding and maintained during tissue homeostasis despite cell turnover and mechanical perturbations. In turn, cell shape also controls tissue function as a regulator of cell differentiation, proliferation and motility. Here we investigate cell shape changes in a model epithelial monolayer. After the onset of confluence, cells continue to divide and change shape over time, eventually to a final steady state characterized by arrested motion and more hexagonal cell shapes. Such monolayer ‘remodeling’ is robustly observed, with qualitatively similar changes in cell shape and dynamics. However, we use quantitative differences in the monolayer remodeling dynamics to reveal underlying order parameters controlling epithelial architecture. For instance, for monolayers formed atop extracellular matrix with varied stiffness, the cell density varies significantly but cell shape dynamics and motile behavior remain similar. Alternately, we find that with varied pharmacological perturbations the monolayer arrests with more elongated cell shapes. Across all experimental conditions this final average cell shape is well correlated to the cell division rate, but oriented cell divisions are insufficient to account for this dependence. Furthermore, we find that inhibition of the cell cycle immediately arrests cell motility and shape change suggesting that active stress from cell divisions contributes significantly to monolayer remodeling. Thus, the architecture and mechanics of epithelial tissue can arise from an interplay between cell mechanics and stresses arising from cell division.