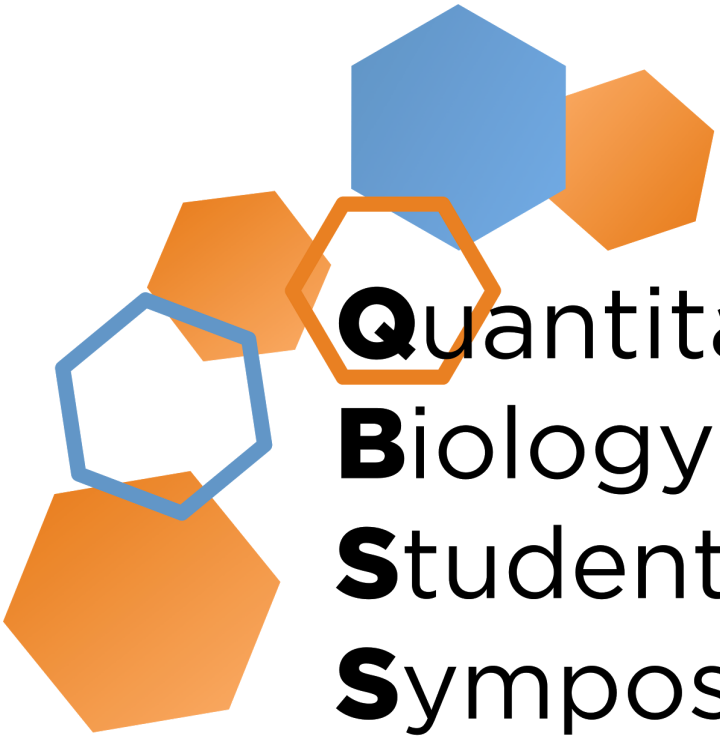


Quantitative Biology Student Symposium

Systems Biology in the Context of Aging and Disease

Jennie Smoly Caruthers Biotechnology Building

May 25, 2017

8:30am-6:00pm

BioFrontiers Institute
IQ BIOLOGY PhD PROGRAM

IQ Biology is generously supported by NSF IGERT Grant #1144807

Welcome to the 2017 IQ Biology Student Symposium on Systems Biology in the Context of Aging and Disease!

We are honored today to be showcasing some of CU's outstanding bioscience students and postdocs at this unique event. These young scientists will share with us their research stories, scientific vision, and transformative ideas for addressing current interdisciplinary bioscience challenges. The students have designed this symposium so that you can get a variety of perspectives in a setting that is small enough to encourage questions and conversation.

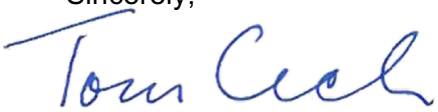
Today promises to be an exciting day of ideas. A major focus area of the IQ Biology program is quantitative biology and collaborative science. Our presenters (including at the poster session) will present ideas from computer science, applied mathematics, engineering, genomics, and chemistry.

This year's event features scientific presentations by 2016 *Butcher Innovation Awards* winners, IQ Biology students, and participants from the first BioFrontiers hackathon. I am also looking forward to the keynote address by David Botstein, Chief Scientific Officer of Calico.

We host this event in a great place. The Jennie Smoly Caruthers Biotechnology Building has been our home for just over five years. It houses many talented, collaborative faculty who are proving that combining different academic disciplines creates opportunities to think in new ways and solve daunting problems.

I would like to thank the IQ Biology students for their hard work organizing this event and I look forward to hearing many excellent presentations. I hope you enjoy this symposium!

Sincerely,



Thomas R. Cech, Ph.D.
Director, IQ Biology
University of Colorado Boulder

Thank you for attending the 2017 IQ Biology Student Symposium on Systems Biology in the Context of Aging and Disease.

This symposium is a biennial event organized by students of the IQ Biology PhD Certificate Program to showcase the interdisciplinary work stemming from the program as well as from across campus. While the focus of the symposium is on aging and disease, we will exhibit cutting edge science from a variety of areas. The symposium will be concluded by an exciting keynote lecture by Dr. David Botstein, the Chief Scientific Officer of Calico.

The Interdisciplinary Quantitative Biology (IQ Biology) PhD Certificate Program was founded in 2010 by the BioFrontiers Institute at the University of Colorado, Boulder, to provide an interdisciplinary environment to train the modern science student. Participants in the IQ Biology Program come from backgrounds ranging from computer science and pure mathematics to ecology and evolutionary biology. Despite our far-reaching backgrounds, we spend our first year of graduate school working together on collaborative projects and classes while also rotating in labs from different disciplines.

We hope you enjoy today's symposium. We have lots of exciting science to discuss and are glad that you've joined us!

Sincerely,

Organizing Committee & Current IQ Bio Students
David Brazel
Patrick Heenan
John Nardini
Cloe Pogoda
Lynn Sanford
Katia Tarasava

Keynote Address

Understanding Cellular Response at the System Level in Yeast

David Botstein, Ph.D.

Chief Scientific Officer

Calico (founded by Google in 2013)

May 25, 2017

3:30 pm

JSCBB Butcher Auditorium

David Botstein, Ph.D. is a prominent geneticist whose advocacy for gene mapping was crucial in laying the groundwork for the Human Genome Project. He is currently the CSO of Calico ("California Life Company"), a biotechnology company focused on longevity and health, established by Google, Inc. in 2013.

David received his Ph.D. from the University of Michigan. He taught at MIT as Professor of Genetics. He served as Science Vice President at Genentech, Inc. and as Chairman of the Department of Genetics at Stanford University's School of Medicine. In 2003 he joined Princeton as the Anthony Evnin Professor of Genomics and Director of the Lewis-Sigler Institute of Integrative Genomics. He established a new graduate program (Quantitative and Computational Biology) and the Lewis-Sigler Fellow program for early career scientists

David is a member of the U.S. National Academy of Sciences and the Institute of Medicine. He has served on the NAS/NRC study on the Human Genome Project (1987-88), NIH Program Advisory Panel on the Human Genome (1989-90), Advisory Council of the National Center for Human Genome Research (1990-1995) and the Advisory Committee to the Director at NIH (2003-2008). His numerous awards include the Eli Lilly Award, the Genetics Society of America Medal, the Allen Award of the American Society of Human Genetics, the Dickson Prize, the Rosenstiel Award, the Gruber Prize in Genetics, the Albany Prize, the Dan David Prize, and the Breakthrough Prize in Life Sciences.

Symposium Schedule

Morning Session — JSCBB A104

8:30 - 9:00 Registration and breakfast

9:00 - 9:15 Welcoming Remarks, Dr. Thomas R. Cech, *Director, IQ Biology, BioFrontiers Institute*

9:15 - 10:15 Butcher Innovation Award Recipient Talks

- 9:15-9:40 Joshua Wheeler and Thomas Vogler
  "*Targeting RNA-protein aggregates in iPSC-derived muscle*"
- 9:45-10:10 Xinfeng Wang and Daniel Gulbranson,
  "*Development of Bivalent Inhibitors Targeting the Interaction of HIV with the Co-receptor CCR5*"

10:15 - 10:30 Coffee break

10:30 - 11:30 IQ Biology Student Talks

- 10:30 - 10:55 Brian Aguado
  "*Serum from Transcatheter Aortic Valve Replacement Patients Reveals Links to Valvular Interstitial Cell Activation*"
- 11:00 - 11:25 Ryan Langendorf
  "*Strange loops in ecology: Identifying causal drivers across the organizational scales of a host-parasite community*"

Lunch — JSCBB B115

11:30 - 1:00 Lunch & Professional Development Panel
Life beyond the bench...Career Pathways for Scientists!

- Adam Richards, *Instructor and Data Scientist, Galvanize*
- Ginny Orndorff, *Executive Director and CEO, CID4*
- John Sanderson, *Director of Science, The Nature Conservancy*

Afternoon Session — JSCBB A104

1:00 - 2:00 Butcher Innovation Award Recipient Talks

- 1:00-1:25 Jeffrey Moore and Cassi Estrem
  "*Flexible Filaments: Modeling a Realistic Cytoskeleton"*
- 1:30-1:55 Jonathan Rubin and Joseph Azofeifa
  "*Determining Gene Networks affected by Chemotherapeutics*"

2:00 - 2:30 Hackathon Presentations

- Mary Allen
- Hamidreza Chitsaz
- Ivo Georgiev

2:30 - 3:30 Poster Session & Coffee *[outside of room A104]*

Keynote Address — JSCBB Butcher Auditorium

3:30 - 5:00 Dr. David Botstein, *Chief Scientific Officer, Calico*
"*Understanding Cellular Response at the System Level in Yeast*"

5:00-6:00 Reception

Poster Session

Poster #1	**Mary Allen**	*Molecular Biology of Down syndrome*
Poster #2	**David Brazel**	*GSCAN Exome: Rare Variants, Alcohol Use and Smoking*
Poster #3	**Elizabeth Delesky**	*Biomimetic Antifreeze Polymers for Cryopreservation*
Poster #4	**Patrick Heenan**	*Hidden Dynamics in the Unfolding of individual bacteriorhodopsin proteins*
Poster #5	**Taisa Kushner**	*Rapid Evaluation to Prevent Dangerous Regimens for an Artificial Pancreas Controller*
Poster #6	**Laura Maguire**	*Mechanisms of diffusion in nuclear pore complex mimics*
Poster #7	**Justin Moser**	*Cell cycle commitment: Exploring the when, and how of the P-Q decision*
Poster #8	**John Nardini**	*Investigation of a Structured Fisher's Equation with Applications in Biochemistry*
Poster #9	**Katie Rainey**	*Mimicking the Nuclear Pore Complex with PEG Hydrogels*
Poster #10	**Nadia Sampaio**	*Systemic genome instability in heterozygous yeast diploids*
Poster #11	**Lynn Sanford**	*Characterizing zinc signaling in hippocampal neurons*
Poster #12	**Katia Tarasava**	*Developing a predictive method for tunable control over gene expression based on CRISPR interference*
Poster #13	**Cierra Walker**	*Manipulating fibroblast mechanical memory with photodegradable PEG hydrogels*
Poster #14	**Jacqueline Wentz**	*Pattern Formation in the Longevity-Related Expression of Heat Shock Protein-16.2 in Caenorhabditis elegans*
Poster #15	**Chen Yang**	*Single-cell analysis of signaling network rewiring in drug-tolerant melanoma cells*
Poster #16	**Cassidy Thompson**	*Platypus - A flexible Linux-based educational platform*

Abstracts

Oral Presentations ***9:00 - 11:30am*** ***JSCBB A104***

Development of Bivalent Inhibitors Targeting the Interaction of HIV and the Co-receptor CCR5

Xinfeng Wang, *Postdoctoral Fellow, Department of Chemistry and Biochemistry, CU Boulder*
Daniel Gulbranson, *Graduate Student, Department of Molecular, Cellular & Developmental Biology, CU Boulder*

Glycosylation is a common modification that is capable of modulating the biophysical and biological properties of proteins. But despite many decades of research effort, the question of how best to exploit glycosylation to improve the performance of therapeutic proteins still remains largely unanswered. This deficiency in knowledge stems mainly from the complex and heterogeneous nature of protein glycosylation. In order to develop optimal protein based therapeutics, it is necessary to better understand protein glycosylation. The objective of our research is to investigate the link between O-linked glycans and various biophysical and biological properties of CCL5 (also known as RANTES), with particularly close attention being paid to glycoforms with decreased inflammatory properties. The findings from the proposed research is expected to lead to the development of useful strategies to improve the properties of CCL5 as an HIV-1 inhibitor and other therapeutic proteins that are important for human health.

Formation of amyloid-like TDP-43 myo-granules during early myogenesis

Joshua Wheeler, Graduate Student, *Department of Chemistry and Biochemistry, CU Boulder*
Thomas Vogler, *Graduate Student, Department of Molecular, Cellular & Developmental Biology, CU Boulder*
(Additional Authors: Eric Nguyen, Michael Hughes, Nicole Dalla Betta, Philip Wong, Paul Taylor, Aaron Johnson, David Eisenberg, Bradley Olwin, Roy Parker)

The mechanisms governing mRNA localization in skeletal muscle are unknown. TDP-43 is an RNA-binding protein that aggregates in neuromuscular disease yet little is known about the aggregation properties and function of TDP-43 in skeletal muscle. Transcriptome-wide crosslinking and immunoprecipitation in differentiating myotubes reveals TDP-43 binds to mature UG-rich myogenic mRNA transcripts. Strikingly, in contrast to neurons where TDP-43 often binds to introns, a majority of the TDP-43 binding in myotubes is in exons. Moreover, TDP-43 redistributes from the nucleus to the cytoplasm during early myogenesis both in cell culture models and in wild type mice suggesting it is bound to and travels with newly synthesized muscle specific mRNAs. Biochemical analysis during differentiation demonstrates TDP-43 is a component of a SDS-resistant higher order RNP assembly, which we term myo-granules. TDP-43 myo-granules stain positive with antibodies specific for amyloid oligomers suggesting they could have amyloid-like features. Finally, in a mouse model of Multisystem Proteinopathy – a neuromuscular disease characterized by the accumulation of TDP-43 aggregates in skeletal muscle – we observe muscle regeneration and cytoplasmic

TDP-43 myo-granule formation is increased. Thus, we propose the formation of TDP-43 amyloid-like myo-granules may regulate and localize specific mRNAs during normal skeletal muscle myogenesis. Moreover, the amyloid-like nature of the myo-granules suggests they might be a precursor to pathological amyloid formation of TDP-43 in degenerative neuromuscular disease.

Serum from Transcatheter Aortic Valve Replacement Patients Reveals Links to Valvular Interstitial Cell Activation

Brian Aguado, *Postdoctoral Fellow, Chemical and Biological Engineering, CU Boulder*
(Additional Authors: Katherine B. Schuetze, Joseph C. Grim, Timothy A. McKinsey, and Kristi S. Anseth)

Background: Transcatheter Aortic Valve Replacement (TAVR) has emerged as a transformative treatment for aortic valve stenosis. However, the impact of TAVR on the composition of serum factors mediating valve fibrosis are unknown. Here, valvular interstitial cells (VICs) seeded on poly(ethylene glycol) (PEG) hydrogels were treated with healthy, pre-TAVR, and post-TAVR patient serum to assess myofibroblast activation as a function of serum factors.

Methods: Soft PEG hydrogels that are known to maintain the quiescent VIC phenotype were fabricated using thiol-ene chemistry. Serum was collected from patients with IRB approval before undergoing TAVR and 1 month post-TAVR. Media samples containing 1% serum were used to treat VICs seeded on hydrogels for 24 hours. Healthy patient serum and serum supplemented with transforming growth factor beta (TGF-b, 10 ng/mL), a myofibroblast activator, served as controls. After treatment, cells were immunostained for alpha smooth muscle actin (a-SMA) stress fibers and imaged.

Results: A majority of VICs retain their quiescence state (no a-SMA stress fibers) on hydrogels when cultured with healthy serum (30.9 +/- 1.3% activated), and become more activated (a-SMA stress fibers present) with TGF-b treatment (68.1 +/- 1.2%). For all four patients tested (Patients A-D), VICs showed increased activation in pre-TAVR serum relative to healthy serum (A: 60.9 +/- 4.4%, B: 49.0 +/- 2.0%, C: 60.8 +/- 1.9%, D: 48.3 +/- 3.2%). VICs cultured in post-TAVR serum showed reduced activation relative to pre-TAVR serum from the same patient (A: 45.8 +/- 2.4%, B: 36.6 +/- 1.5%, C: 32.5 +/- 1.6%, D: 31.6 +/- 4.9%). When male and female data were pooled together, VICs cultured in post-TAVR serum from male patients showed a decrease in activation (32.1 +/- 3.4%) relative to female post-TAVR serum (41.2 +/- 5.3%).

Conclusions: TAVR patient serum likely contains multiple secreted factors that modulate myofibroblast activation and may impact valve tissue remodeling. Male and female post-TAVR serum may also vary in protein composition. We seek to characterize the secreted factors in serum using DNA aptamer arrays as a strategy to identify activators of the pathogenic myofibroblast phenotype. In sum, PEG hydrogels serve as a useful platform to unambiguously test serum protein effects on VIC activation.

Strange loops in ecology: Identifying causal drivers across the organizational scales of a host-parasite community

Ryan Langendorf, *Graduate Student, Environmental Sciences and IQ Biology Programs, CU Boulder*

Community ecologists have historically treated properties of ecosystems as entirely emergent rather than also causal. In doing so ecologists have assumed a community's structure cannot drive the abundances of constituent species as part of a process of maintenance. Community structures have therefore been correlated with and predicted by models of constituent species dynamics without consideration to the roles they may play in them. Testing this assumption experimentally is challenging because an ecosystem property cannot be altered without also changing the constituent population abundances. Fortunately, there is now substantial evidence that the mathematics underlying state space reconstructions of coupled time series can infer causal interactions in nonlinear systems. I have applied this Empirical Dynamic Modeling (EDM) framework to a rodent-ectoparasite community's abundances and interactions observed at ten locations in Slovakia between 1983 and 2001. A previous analysis of this system found that properties of the bipartite host-parasite networks could be accounted for solely by the abundances of the hosts and parasites. Their conclusion that the community's structure was the result of neutral interactions assumed those structures could not have caused the abundances of the hosts or parasites. Only 3 hosts, 7 parasites, and 3 network properties met the assumptions of EDM, but within this subcommunity I identified causal drivers both within and across organizational scales. This suggests the utility in applying these kinds of nonlinear state-space reconstruction techniques more broadly, and the importance of causal feedbacks across organizational scales in the creation and maintenance of ecosystems.

Professional Development Workshop 11:30 - 1:00 pm JSCBB B115

Speaker Biographies

Ginny Orndorff
Executive Director, Colorado Institute for Drug, Device, and Diagnostic Development

Ginny Orndorff joined CID4 in February 2015. She has over 30 years of bioscience company management experience. She also serves as Vice Chair of the Colorado BioScience Association and acting CEO of SixOne Solutions, LLC, a University of Colorado Anschutz Medical Campus spin-off company working on novel cancer drugs. Ginny was a founder of Evolutionary Genomics, Inc. of Longmont, CO and was its CEO for 10 years, continuing today on its Board of Directors. Prior to that, she was CEO of GenoPlex of Denver, CO. She also served as Director of Technology/Business Development for NeXstar of Boulder and Director of Biotechnology Programs at the Colorado Advanced Technology Institute. Ginny began her bioscience career as a lab supervisor at Genex in Gaithersburg, MD. She received a Bachelor's Degree in Biology from the University of California Santa Cruz, a Master's in Microbiology from California State University San Jose, and an MBA from Loyola College in Baltimore.

Adam Richards
Lead Instructor and Data Scientist, Galvanize

Having worked in medicine and ecology, Adam has used data science in a wide variety of problem scenarios. Large, complex datasets are common in these fields and computational biologists, like Adam, make frequent use of machine learning along with high performance computing to make sense of these data. His work has taken many themes from graph theory to customized Bayesian models.

Currently, Adam is the lead instructor for a data science immersive program at Galvanize. He and his team teach across the spectrum of data science including: conventional statistics, machine learning, databases, AWS, Spark and application building.

John Sanderson
Director of Science, The Nature Conservancy

John Sanderson is Director of Science for the Nature Conservancy of Colorado. John leads a staff of scientists who work on a range of conservation challenges, including determining how much water is enough for endangered fish in the Yampa River, measuring the effects of fires in Colorado's Front Range forests, planning for sustainable grazing on hundreds of thousands of acres on the Great Plains, and adapting conservation strategies to a changing climate. After earning his BS in Engineering from Purdue University and an MS in Botany from the University of Vermont, John got his start in Colorado in 1994 doing field inventory and conservation planning for the Colorado Natural Heritage Program. He later earned his PhD in the Graduate Degree Program in Ecology doing research on the hydrology, vegetation, and conservation of intermountain playa wetlands in Colorado's San Luis Valley. John is passionate about figuring out how we conserve species and ecosystems as Colorado's population explodes and our climate continues to change.

Poster Abstracts ***2:30-3:30pm*** ***Outside of JSCBB A104***

Poster #1

Molecular biology of Down Syndrome

Mary Allen**,** *Research Assistant Professor, BioFrontiers Institute, CU Boulder*

Down syndrome is a common condition in which individuals have one extra copy of chromosome 21. This results in lower IQ and distinctive facial features. We are using short-read sequencing to understand the molecular response to Down syndrome. Using genome sequencing we show that the de novo mutation rate is not increased in down syndrome. Additionally we use GRO-seq and RNA-seq to show potential molecular effects of an extra copy of chromosome 21.

Poster #2

GSCAN exome: Rare variants, alcohol use and smoking

David Brazel, *Graduate Student, Molecular Cellular and Developmental Biology, and IQ Biology, CU Boulder*

The use and abuse of alcohol and nicotine has a significant impact on public health. Twin and family studies show that these behaviors have a significant genetic component. Genetic association studies have discovered common variants associated with alcohol and nicotine. The exome chip portion of the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) was formed to examine the association between rare nonsynonymous variants and alcohol and nicotine use, by aggregating and meta-analyzing studies using exome sequencing or genotyping arrays with substantial rare exonic content. We performed extensive quality control and phenotype standardization, followed by rare variant association analyses for five phenotypes: smoking initiation (total N=164,142), cigarettes per day (total N=75.493), age of initiation of smoking (total N=64,616), pack years (total N=72,909), and drinks per week (total N=139,103). We find significant novel associations between cigarettes per day and a variant in the gene STARD3 and two intergenic variants on chromosome 19. The variant in STARD3 failed to replicate in two independent datasets: the CHD Exome+ Consortium (N=17.789, p=0.94) and the Consortium for Genetics of Smoking Behaviour (N=28,583, p=0.84). Replication is pending for the intergenic variants. We also replicated the classic association of ADH1C with alcohol use in both single variant and gene based tests. The GSCAN project is also performing a GWAS analysis of alcohol and tobacco use phenotypes, with sample sizes currently as high as 238,045 individuals for smoking initiation. We are currently meta-analyzing these results and expect additional studies to contribute.

Poster #3

Biomimetic antifreeze polymers for cryopreservation

Elizabeth Delesky, *Graduate Student, Civil,Environmental and Architectural Engineering, CU Boulder*

Today, over 120,000 Americans are waiting for organ transplants, and given that geographical proximity to donation sites plays a major role in determining to whom the United Network for Organ Sharing sends an organ, many patients in critical conditions will not be considered. For organs such as the heart and lungs, this distance is reduced to a radius of 4-6 hours due to low preservation time. By increasing preservation time, larger radii can be considered and patients with the highest need for organ transplants can receive aid, reducing the number of deaths due to waiting. The primary objective of this research is to design and synthesize biomimetic antifreeze polymers (BAPs) that mimic the behavior of antifreeze proteins (AFPs) naturally found in plants, fish, insects, and bacteria to improve the longevity of human organs in transit for transplantation. Synthetic BAPs offer the potential for rapid large-scale production of small-concentration, nontoxic, low-cost polymers with antifreeze behavior without the risk of cell damage. Only recently, researchers have produced the first BAPs using polyvinyl alcohol (PVA) to replicate activity of antifreeze glycoproteins. These PVA BAPs are too large, their random-coil structures are too dissimilar from native AFPs, and PVA side groups were not modified to mimic the functionality or helical regularity of AFPs. Therefore, only low to moderate ice inhibition activity was observed. Furthermore, while PVA exhibits little to no cytotoxicity, it is not biodegradable in the human body, potentially giving rise to issues with transplantation. With an ultimate goal of creating biocompatible BAPs that can be readily used for cryogenic technologies and counter the limitations noted with BAPs from PVA, the primary objective of this work is to design and synthesize low-cost, non-toxic synthetic BAPs based on poly(lactic-co-glycolic acid) (PLGA) and poly(isocyanopeptide) (PICP) that explicitly mimic the thermal hysteresis behavior (i.e., freezing-point depression) of natural AFPs. This preliminary research will look at the synthesis and characterization of PLGA and PICP polymers and their effect on freezing point depression in biologically relevant solutions.

Poster #4

Hidden dynamics in the unfolding of individual bacteriorhodopsin proteins

Patrick Heenan, *Graduate Student, Physics and IQ Biology, CU Boulder*

Protein folding occurs as a set of transitions between structural states within an energy landscape. An oversimplified view of the folding process emerges when transiently populated states are undetected because of limited instrumental resolution. Using force spectroscopy optimized for 1-μs resolution, we reexamined the unfolding of individual bacteriorhodopsin molecules in native lipid bilayers. The experimental data reveal the unfolding pathway in unprecedented detail. Numerous newly detected intermediates — many separated by as few as 2–3 amino acids — exhibited complex dynamics, including frequent refolding and state occupancies of <10 μs. Equilibrium measurements between such states enabled the folding free-energy landscape to be deduced. These results sharpen the picture of the mechanical unfolding of membrane proteins and, more broadly, enable experimental access to previously obscured protein dynamics.

Poster #5

Rapid Evaluation to prevent dangerous regimens for an artificial pancreas controller

Taisa Kushner**,** *Graduate Student, Applied Mathematics and IQ Biology, CU Boulder*

The Artificial Pancreas (AP) Controller presents one of the most promising treatment strategies for diabetes. In order for an AP controller to work effectively, it must evaluate timing and dose schedules for insulin in real-time. In this work, we determine a method to efficiently characterize all eigenvalues of the nonlinear two-delay glucose-insulin system and enable the rapid evaluation of dosing schedules for the AP controller by coupling the Gauss-Lucas theorem with the Lambert W function.

Poster #6

Mechanisms of diffusion in nuclear pore complex mimics

Laura Maguire, *Graduate Student, Physics, CU Boulder*

Few cellular processes require such intricate active control as transport through the nuclear envelope. The nuclear pore complex (NPC) facilitates all transport, preventing most macromolecules from crossing the envelope while allowing the passage of transport factors (TFs) and their cargo. While the basic biochemical interactions of transport are well-understood, the detailed mechanism remains a topic of significant debate. We create tunable mimics of the NPC using PEG hydrogels filled with FG nucleoporins (FG nups), the intrinsically disordered proteins that line the NPC channel in vivo. Using fluorescence microscopy and single-molecule fluorescence spectroscopy, we measure TF diffusion through the NPC mimics. Modeling based on our results suggests two possible mechanisms of TF diffusion through the nuclear pore. Both mechanisms rely on the flexible nature of the disordered FG nups and on the transient nature of FG nup-TF interactions. Our model makes selectivity predictions that will be directly testable in our experimental setup. We aim to distinguish between these possible mechanisms and to tune the mimic's parameters to maximize the rate of passage of TFs while inhibiting the passage of other inert molecules.

Poster #7

Cell cycle commitment: Exploring the when, and how of the P-Q decision

Justin Moser, *Graduate Student, Chemistry and Biochemistry, CU Boulder*

Broadly speaking, cells face one of two fates after each division: to commit to another round of proliferation, or to either temporarily or permanently exit the cell cycle. Proper regulation of this decision is essential for normal growth and development, whereas dysregulation of the proliferation-quiescence decision can lead to uncontrolled growth, one of the hallmarks of cancer. By combining time-lapse, live-cell microscopy and fixed cell immunofluorescence, we show that both noncancerous and cancerous cell lines can experience a transient quiescence after each mitosis. We also provide evidence that, contrary to the popular model of the restriction point, cells are born committed to either proliferation or quiescence. Finally, we explore the role that the cell cycle inhibitory protein p21 plays in informing the proliferation-quiescence decision at the end of the previous cell cycle.

Poster #8

Investigation of a structured Fisher's Equation with applications in biochemistry

John Nardini, *Graduate Student, Applied Mathematics and IQ Biology, CU Boulder*

Recent biological research has sought to understand how biochemical signaling pathways, such as the mitogen-activated protein kinase (MAPK) family, influence the migration of a population of cells during wound healing. Fisher's Equation has been used extensively to model experimental wound healing assays due to its simple nature and known traveling wave solutions. This partial differential equation with independent variables of time and space cannot account for the effects of biochemical activity on wound healing, however. To this end, we derive a structured Fisher's Equation with independent variables of time, space, and biochemical pathway activity level and prove the existence of a self-similar traveling wave solution to this equation. We also consider a more complicated model with different phenotypes based on MAPK activation and numerically investigate how various temporal patterns of biochemical activity can lead to increased and decreased rates of population migration. An example or two will be exhibited, as well as an outline for applying this framework to biological data of wound healing.

Poster #9

Mimicking the nuclear pore complex with PEG hydrogels

Katie Rainey, Graduate Student, Physics, CU Boulder

Many cellular processes rely on the transport of molecules between the cytoplasm and nucleus. The nuclear pore complex (NPC) is a small channel in the nuclear envelope that facilitates transport between the nucleus and cytoplasm of eukaryotic cells, and contains many disordered proteins, FG nucleoporins (FG Nups). Notably, the NPC's transport mechanism is selective; though it blocks the transport of small molecules over 5 nm, it allows for cargo-carrying transport factors of much larger size to pass through. Though the key players in selective transport are known, the mechanism behind the NPC's selectivity is not well understood. In our lab, we are trying to understand selective nucleocytoplasmic transport through the NPC by creating PEG hydrogel mimics. Using fluorescence microscopy, we observe the diffusion of two similarly-sized molecules, m-Cherry and a GFP-labeled transport factor (NTF2), through the hydrogel. We expect to see NTF2 pass through at a much faster rate than m-Cherry. Preliminary results have shown selective transport with rapid entry and exit out of the hydrogel, which is often not achieved with other NPC mimics.

Poster #10

Systemic genome instability in heterozygous yeast diploids

Nadia Sampaio, *Graduate Student, Cell and Molecular Biology, Colorado State University*

Loss-of-heterozygosity (LOH), frequently associated with copy number variation (CNV), is a hallmark of cancer cells. However, experimental demonstration of how these chromosomal alterations evolve poses a major scientific challenge. In this study, we employed the highly heterozygous wild yeast diploid JAY270 to qualitatively and

quantitatively characterize LOH genome-wide. We showed that this strain is an ideal model for straightforward visual selection of clones that underwent at least one LOH event at Chr12 (rough colonies) and clones with no known LOH events (smooth colonies), due to a heterozygous missense mutation at the ACE2 gene. Initial PFGE karyotypic analysis of 29 smooth clones (no selected LOH events) showed no indicatives of LOH/CNV in any of them, consistent with what would be expected based on the estimated rate of genome-wide mitotic crossover. In contrast, PFGE of 25 spontaneous rough colony isolates revealed 6 clones with additional unselected chromosomal polymorphisms. This number is much higher than what would be expected based on the estimated rate of genome-wide mitotic crossover, which predicts that only one clone out of the 25 should have one unselected LOH event (6.2 x 10-4 crossovers/genome x 57 cell divisions x 25 clones). Subsequent WGS analysis of the rough colony clones revealed an astonishing total of 27 unselected secondary LOH tracts. These results provided strong evidence that some cells in culture experience multiple genome alterations in a dependent fashion, a process that may reflect a systemic genome instability mechanism. To independently validate this observation and eliminate any possible influence from Chr12 genotype status on genome instability, we built strains individually hemizygous for the CAN1 gene (Chr5) and/or the counter selectable CORE2 cassette. We then measured the rate of individual and coincident double LOH by selecting for resistance to canavanine and/or 5-FOA. We observed double LOH rates 30-100 fold higher than the expected for both events occurring independently of one another, reinforcing the model of punctuated, systemic bursts of genome instability leading to LOH. This effect was observed in two different strain backgrounds (JAY270 and CG379) for two pairs of genomic regions (Chr5/Chr4 and Chr5/Chr13), in all cases with Chr12 remaining unaltered. This uncovered pattern of genome instability resembles recent discoveries in cancer genomes and has an enormous impact on the prevailing model of cancer gradual evolution and, in the case of unicellular organisms, could represent a major driver of genome evolution.

Poster #11

Characterizing zinc signaling in hippocampal neurons

Lynn Sanford, Graduate Student, Chemistry and Biochemistry, CU Boulder

Zinc is an essential ion that is important for many proteins and for some cell signaling processes. In mammalian hippocampal neurons, zinc is released from synaptic vesicles and appears to act as a neurotransmitter. Little is known about the mechanism and function of these zinc signals, although they have been correlated with modulation of signaling pathways and with broader functions of encoding memory. Furthermore, persistently high intracellular zinc levels have been seen in neurodegenerative pathologies including stroke, epilepsy, and Alzheimer's disease. Examining targets and effects of cellular zinc signaling is therefore vital to understanding its endogenous and pathological roles in the brain. We have developed a dissociated hippocampal neuron model system in which we have characterized intracellular and synaptic zinc. Part of this characterization has involved utilizing zinc FRET sensors to visualize and quantify zinc levels and dynamics in neurons at rest and after depolarization. We have also further characterized the downstream targets of zinc signaling through RNA-Seq.

Poster #12

Developing a predictive method for tunable control over gene expression based on CRISPR interference

Katia Tarasava, Graduate Student, *Materials Science and Engineering and IQ Biology, CU Boulder*

Precise control over gene expression is essential for advancing metabolic engineering, as well as general understanding of the global context of cellular regulatory networks. Engineering and optimizing metabolic pathways requires being able to fine-tune expression of multiple genes simultaneously in a precise manner, which is restricted by time-consuming traditional strain engineering methods and a limited number of dose-response promoters. These limitations can be overcome by using CRISPR-based gene repression and activation [1]. In addition to offering multiplex gene regulation, CRISPR interference allows to control the degree of gene expression at the level of transcription. Transcriptional control can be achieved by adjusting the strength of interaction between the guide RNA and target gene through incorporating mismatches into the guide RNA sequence. The number, type and position of mismatches can affect the strength of gRNA binding and consequently, target gene expression [2]. We seek to describe the relationship between gRNA sequence and its effective strength by using an adjustable parameter Markov model. The model can be trained on real expression data and be used as a tool for predicting and precisely controlling the level of transcription for a given gene. The application of this method has the potential to greatly simplify and reduce the cost of strain engineering, as well as provide insight into fundamental properties of metabolic networks

Poster #13

Manipulating fibroblast mechanical memory with photodegradable PEG hydrogels

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Aortic valve stenosis (AVS) is caused by fibrosis of the aortic heart valves. As many as 25% of adults over 65 suffer from valvular stenosis and currently, the only treatment is surgical replacement. Activation of valvular interstitial cells (VICs) to myofibroblasts and their persistence play a key role in AVS. In healthy tissue, VICs transiently activate to myofibroblasts following injury. However, chronic exposure to stiffened mechanical environment prevents reversal of myofibroblasts to a quiescent VIC state, resulting in persistently activated myofibroblasts. This in vivo time-dependent response to stiffness implies VICs possess a mechanical memory of their past environments. Unfortunately, little is known about how persistently activated myofibroblasts maintain their mechanical memory of stiff environments. In the past, the development of in vitro models for AVS using standard cell culture practices has been challenging because the high elastic modulus of tissue culture polystyrene causes VICs to persistently activate to myofibroblasts. However, the healthy, quiescent VIC phenotype can be maintained by using a photo-tunable poly(ethylene glycol) (PEG) hydrogel cell culture platform. These materials allow for precise control of the cellular mechanical environment because the mechanical properties can easily be tuned to mimic physiological conditions. By using the PEG hydrogel system, I identified a time-dependent mechanical dose on stiff

hydrogels that triggers VICs to transform from transiently activated myofibroblasts to persistently activated myofibroblasts. After a short time period (2 -5 days), activated VICs can revert back to a quiescent state when switched from stiff to soft elastic moduli. However, after VICs are exposed to stiff environments for a long period of time (>7 days), the cells no longer are able to return to the quiescent state. I plan to probe the signaling pathways associated with mechanical memory by using transcriptome analysis. Potential pathways will be validated using siRNAs and/or pharmacological inhibitors. If we can understand how VICs maintain mechanical memory, targeting members of the discovered pathways could provide a much-needed non-surgical treatment for individuals suffering from AVS.

Poster #14

Pattern Formation in the Longevity-Related Expression of Heat Shock Protein-16.2 in Caenorhabditis elegans

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Isogenic populations of Caenorhabditis elegans exposed to heat shock have lifespans ranging from 3 to 16 days. This highly variable lifespan is correlated with the expression of a small heat shock protein (hsp-16.2) within intestinal cells. Although evidence has shown that the heat shock response is triggered by the insulin-like signaling pathway, the exact mechanism is unknown. Intriguingly, the cell-specific expression of hsp-16.2 is dependent on the spatial location of the cell along the length of the worm. We hypothesize that the patterned expression of hsp-16.2 is caused by a diffusion-driven instability within the pseudocoelom, or fluid-filled cavity, that borders the intestinal cells in C. elegans. This instability is due to the interactions between two classes of insulin like peptides that serve antagonistic roles. To test this hypothesized mechanism, we develop a mathematical model of the system and compare output from the model to experimental data on heat shock protein expression. Furthermore, we use the model to gain insight on possible biological parameters in the system. The model presented is capable of producing patterns similar to what is observed experimentally and provides a first step in mathematically modeling aging-related mechanisms in *C. elegans*.

Poster #15

Single-cell analysis of signaling network rewiring in drug-tolerant melanoma cells

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Spontaneous genetic mutations allow an initially drug-sensitive population of cancer cells to acquire a drug-resistant phenotype. However, little is known about how drug-sensitive cells first evade drug action and survive in the presence of drug, referred to as “drug tolerance”, a crucial step on the road to resistance. Here we use single-cell time-lapse microscopy to study the early signaling response of BRAFV600E melanoma cells to clinical BRAF inhibitors. By multiplexing live-cell sensors for MAPK signaling, PI3K/AKT signaling, and cell-cycle progression, we show that there is a small population of melanoma cells that are initially drug-sensitive and enter quiescence in response to drug, but then re-wire their signaling networks within a few days of drug treatment to acquire drug-tolerance and resume proliferation. ERK activity is immediately reduced

after BRAF inhibitor treatment in all cells, but re-activates in the sub-population of drug-tolerant cells prior to cell-cycle re-entry. We also find that while AKT signaling is moderately active in untreated BRAFV600E melanoma cells, AKT activity increases in all cells upon treatment with BRAF inhibitors. Combination therapy, using a BRAF inhibitor and an ERK or AKT pathway inhibitor, results in fewer drug-tolerant cells and more cell killing. Our results suggest that acquisition of drug tolerance can occur within 2-3 days, long before genetic mutations arise causing bona fide drug resistance. Knowledge of how cells rewire signaling networks in response to targeted therapies may lead to the development of combination of cancer therapies that can reduce the emergence of drug resistance in cancer.

Poster #16
Platypus - A flexible Linux-based educational platform
Cassidy Thompson, *Web Developer, BioFrontiers Institute*

Platypus, a BioFrontiers hosted web resource, is an educational platform designed to create an accessible teaching method for complex topics that require interaction with a Linux environment. Platypus was developed and created at the 2017 BioFrontiers Hackathon to allow distribution of existing BioFrontiers training courses, facilitate the development of new educational content, and include documentation for independently using the software and various deployment methods. As a result, these courses, which were previously tied to the BioFrontiers scientific computing environment, will now be available through a placeless learning experience that can be broadly shared with outside users and institutions. These training resources will be prepared for deployment on diverse cloud resources with a common user experience. Additionally, a Docker deployment path will be available to ensure the resource is accessible from individual workstations if cloud resources are cost-prohibitive or unavailable. The central focus of the platform is to create a web accessible system for uploading modules that will be integrated into the platform and documented to allow teaching of diverse subjects and software tools. Education modules can include video, supporting documents, man pages, and output files from installed software.

Flexible Filaments: Modeling a Realistic Cytoskeleton
Jeffrey Moore, *Graduate Student, Physics, CU-Boulder*
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Microtubules are the most rigid filaments that compose the cytoskeleton of the cell, with persistence lengths on the order of millimeters. Since the length scale of a cell is on the order of micrometers, microtubules are often modeled as perfectly rigid rods for simplicity. However, there exist many regimes in which the buckling and bending of microtubules play a key role for cytoskeletal dynamics, and it is therefore important to properly model the flexibility of cytoskeletal filaments in these systems. I will discuss one such model used in our simulations as well as review preliminary results of dynamics captured by this model.

Transcriptional Regulation of Chemotherapy Resistance in Cancer
Joseph Azofeifa, *Graduate Student*[1]*, Computer Science and IQ Biology, CU Boulder*
Jonathan Rubin, *Graduate Student, Chemistry and Biochemistry, CU Boulder*

Transcription, as mediated by transcription factors (TFs), is a key regulatory step in the expression of functional gene products. The study of this process on a global scale has been limited by the availability of sequencing techniques that directly measure transcription as opposed to steady-state RNA. Using Global Run On with sequencing (GRO-Seq), a technique that captures nascent transcripts, we discovered global changes in transcription of genes and enhancer RNAs (eRNAs) between resistant and non-resistant cancer cells. Using computational approaches, we discovered that the response to chemotherapeutics in resistant cancer cells is a transcriptionally active process mediated by the activation of specific TFs. These findings provide insights into gene programs necessary for the acquisition of chemotherapy resistance and potential therapeutic targets for combating cancer cell resistance to chemotherapy.

[1] Graduated May 2017