Pennsylvania Muscle Institute
Annual Retreat and Symposium

Perelman School of Medicine
University of Pennsylvania

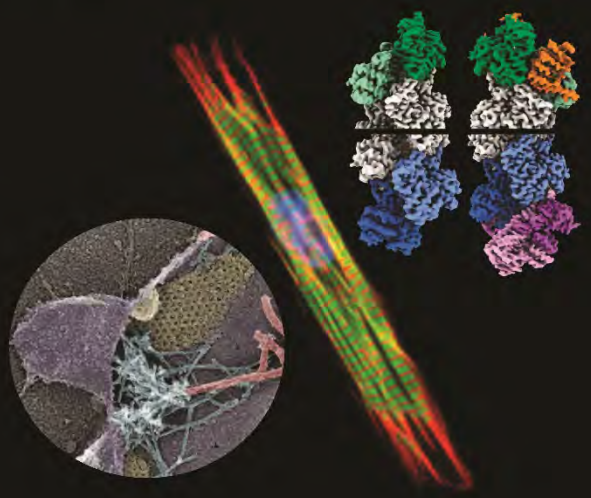
Celebrating 50 Years
Muscle, Motors, and the Cytoskeleton

October 16, 2023

Gaulton Auditorium and Lobby
Biomedical Research Building (BRB II/III)
421 Curie Blvd., 1st Floor

Jean and Joseph Sanger Lecture in Muscle Biology:

James A. Spudich, PhD
Stanford University
"Myosin, the exquisite nanomachine: From basic science to biotech to medicines"



Andrew Somlyo Honorary Lectures:

Jennifer Lippincott-Schwartz, PhD
HHMI Janelia Research Campus
"How the cytoskeleton controls the structure and dynamics of the endoplasmic reticulum"

Andrew P. Carter, PhD
MRC Laboratory of Molecular Biology
"Cargo transport by dynein/dynactin"

Penn Speakers:

Sharlene M. Day, MD
Division of Cardiovascular Medicine
"Mechanisms of Myosin Binding Protein C Mutations in Hypertrophic Cardiomyopathy"

Roberto Dominguez, PhD
Department of Physiology
"Structural-Functional Mechanisms Controlling Actin Filament Barbed and Pointed End Dynamics"

Hansell H. Stedman, MD
Department of Surgery
"Gene Therapy for Inherited Muscle Disease: A Glimpse of the Summit Ridge from Everest Base Camp"

Xingyuan Fang (Svitkina Lab)
Department of Biology
"Mechanism of branched actin assembly in microtubule- and APC-dependent manner"

Adam Fenton (Holzbaur and Jongens Labs)
Department of Physiology
"FMRP-associated protein synthesis locally determines mitochondrial organization in neurons"

Jennifer Petrosino, PhD (Prosser Lab)
Department of Physiology
"The Hitchhiker's Guide to the Myocyte: Active transport of tRNAs facilitates distributed protein synthesis"

Qing Tang, PhD (Lakadamyali Lab)
Department of Physiology
"Insight into cytoskeleton sorting from microtubule detyrosination"

Scan for schedule

www.med.upenn.edu/pmi

Poster session for all attendees

Pennsylvania Muscle Institute
Annual Retreat and Symposium 2023

"Celebrating 50 Years of Muscle, Motors, and the Cytoskeleton"

Monday, October 16, 2023

Location: BRB Gaulton Auditorium & Lobby,
Biomedical Research Building (BRB II/III),
421 Curie Blvd., 1st Floor, Philadelphia, PA 19104

Sponsored by the Physiological Society of Philadelphia and the Pennsylvania Muscle Institute

www.med.upenn.edu/pmi

8:30 – 9:00am	**Registration Check-in, Poster Setup, Breakfast, Coffee** Location: BRB Gaulton Auditorium & Lobby* **Table seating available in BRB 14th Floor Lounge*
9:00 – 9:15am	***Welcome*** **E. Michael Ostap, PhD** Director, Pennsylvania Muscle Institute Professor of Physiology University of Pennsylvania **Jonathan A. Epstein, MD** Executive Vice Dean and Chief Scientific Officer, Perelman School of Medicine at the University of Pennsylvania Senior Vice President and Chief Scientific Officer, University of Pennsylvania Health System William Wikoff Smith Professor of Medicine (Cardiology) and Cell and Developmental Biology University of Pennsylvania
9:15 – 9:30am	***50 Years of Progress at the PMI*** **E. Michael Ostap, PhD** Director, Pennsylvania Muscle Institute Professor of Physiology University of Pennsylvania

9:30 – 10:15am	***Andrew P. Somlyo Honorary Lecture*** **Jennifer Lippincott-Schwartz, PhD** Senior Group Leader and Head of Janelia's 4D Cellular Physiology, HHMI Janelia Research Campus *"How the cytoskeleton controls the structure and dynamics of the endoplasmic reticulum"*
10:15 – 10:30am	**Adam Fenton (Holzbaur and Jongens Labs)** University of Pennsylvania *"FMRP-associated protein synthesis locally determines mitochondrial organization in neurons"*
10:30 – 11:00am	**Coffee Break, Posters**
11:00 – 11:45am	***Andrew P. Somlyo Honorary Lecture*** **Andrew P. Carter, PhD** Investigator, MRC Laboratory of Molecular Biology *"Cargo transport by dynein/dynactin"*
11:45am – 12:15pm	**Roberto Dominguez, PhD** William Maul Measey Presidential Professor of Physiology, University of Pennsylvania *"Structural-Functional Mechanisms Controlling Actin Filament Barbed and Pointed End Dynamics"*
12:30 – 1:30pm	**Lunch, Posters** Location: BRB Lobby* **Table seating available in BRB 14th Floor Lounge*
1:30 – 1:45pm	**Xingyuan Fang (Svitkina Lab)** University of Pennsylvania *"Mechanism of branched actin assembly in microtubule- and APC-dependent manner"*
1:45 – 2:00pm	**Qing Tang, PhD (Lakadamyali Lab)** University of Pennsylvania *"Insight into cytoskeleton sorting from microtubule detyrosination"*
2:00 – 2:30pm	**Hansell H. Stedman, MD** Professor of Surgery, University of Pennsylvania *"Gene Therapy for Inherited Muscle Disease: A Glimpse of the Summit Ridge from Everest Base Camp"*
2:30 – 3:00pm	**Coffee Break, Posters**
3:00 – 3:15pm	**Jennifer Petrosino, PhD (Prosser Lab)** University of Pennsylvania *"The Hitchhiker's Guide to the Myocyte: Active transport of tRNAs facilitates distributed protein synthesis"*

3:15 – 3:45pm	**Sharlene M. Day, MD** Presidential Associate Professor of Medicine & Director, Translational Research, Division of Cardiovascular Medicine University of Pennsylvania *"Mechanisms of Myosin Binding Protein C Mutations in Hypertrophic Cardiomyopathy"*
3:45 – 4:00pm	***Introduction: Jean and Joseph Sanger Lecture in Muscle Biology*** **E. Michael Ostap, PhD**
4:00 – 4:45pm	***Jean and Joseph Sanger Lecture in Muscle Biology*** **James A. Spudich, PhD** Douglass M. and Nola Leishman Professor of Cardiovascular Disease, Stanford University *"Myosin, the exquisite nanomachine: From basic science to biotech to medicines"*
4:45 – 5:45pm	**Reception, Posters** Location: BRB Lobby
5:45 – 6:00pm	**Farewell, Posters take-down** Location: BRB Lobby

Pennsylvania Muscle Institute

Perelman School of Medicine
University of Pennsylvania

The Pennsylvania Muscle Institute (PMI) is an internationally renowned center for muscle and motility research supported by Penn Medicine with a mission to:

- Discover the mechanisms of muscle function, muscle disease and motile biological systems through innovative and cross-disciplinary research, and to apply these discoveries to new therapies,
- Develop state-of-the art technologies for the study of muscle and motile systems,
- Provide education and training in muscle biology and motility to scientists, physicians, and students.

Research is conducted by its more than 60 laboratories using biophysics, biochemistry, genetics, physiology and ultrastructure to understand cell migration and intracellular transport, molecular motors, cell division, muscle contraction and development, muscle pathologies and therapies targeted to muscle disease. We are prominent in technological and methodological development for these investigations especially in advanced light microscopy, structural spectroscopy, nanotechnology, biochemical kinetics, image processing, molecular biology, and viral gene targeting. Extramural grants, seminars, symposia, and journal clubs are uniquely initiated and supported by the PMI. Additionally, the PMI sponsors vigorous graduate and post-doctoral training activities, including a NIAMS-supported training program in "Muscle Biology and Muscle Disease."

For questions or inquiries about PMI membership, please contact:

E. Michael Ostap, Ph.D.
Director, PMI
Professor of Physiology
Email: ostap@pennmedicine.upenn.edu

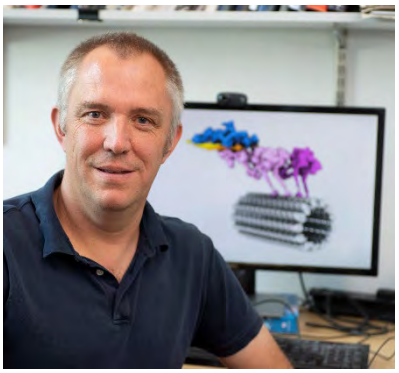
Benjamin L. Prosser, Ph.D.
Associate Director, PMI
Associate Professor of Physiology
Email: bpros@pennmedicine.upenn.edu

Pennsylvania Muscle Institute (PMI)
Perelman School of Medicine at the University of Pennsylvania
700A Clinical Research Building
415 Curie Blvd. Philadelphia, PA 19104

Please visit our website: http://www.med.upenn.edu/pmi/

Guest Speaker Biographies

Andrew P. Somlyo Honorary Lectures



Andrew P. Carter, PhD

Investigator, MRC Laboratory of Molecular Biology

Dr. Andrew Carter studied Biochemistry at Oxford University. He obtained his PhD with Dr. Venki Ramakrishnan at the MRC Lab of Molecular Biology (LMB), working on how antibiotics bind the ribosome. He was part of the team which contributed to Venki's 2009 Nobel Prize. In 2003, Dr. Carter joined the lab of Professor Ron Vale at UCSF to investigate the structural mechanism for dynein binding to the microtubule. He set up his own lab, in the LMB Structural Studies division in 2010, where he and his team have used X-ray crystallography, cryo-EM and many other approaches to understand how cytoplasmic dynein and its cofactor dynactin select and transport cargos. Dr. Carter is a fellow of Clare College, a Wellcome Investigator, and a member of EMBO. Dr. Carter was recently awarded the British Society of Cell Biology (BSCB) Hooke Medal award for 2023.

Jennifer Lippincott-Schwartz, PhD

Senior Group Leader and Head of Janelia's 4D Cellular Physiology, HHMI Janelia Research Campus

Dr. Jennifer Lippincott-Schwartz is a Senior Group Leader at the Howard Hughes Medical Institute's Janelia Research Campus. She has pioneered the use of green fluorescent protein technology for quantitative analysis and modelling of intracellular protein traffic and organelle dynamics in live cells and embryos. Her innovative techniques to label, image, quantify and model specific live cell protein populations and track their fate have provided vital tools used throughout the research community. Her own findings using these techniques have reshaped thinking about the biogenesis, function, targeting, and maintenance of various subcellular organelles and macromolecular complexes and their crosstalk with regulators of the cell cycle, metabolism, aging, and cell fate determination. She is an elected member of the National Academy of Sciences, the National Academy of Medicine, the American Society of Arts and Sciences and the European Molecular Biology Organization. She is also a Fellow of The Biophysical Society, The Royal Microscopical Society and The American Society of Cell Biology. Her awards include the E.B. Wilson Medal of the American Society of Cell Biology, the Newcomb Cleveland Prize of the American Association for the Advancement of Science, the Van Deenen Medal, the Keith Porter Award of the American Society of Cell Biology, the Feodor Lynen Medal, and the Feulgen Prize of the Society of Histochemistry. She co-authored of the textbook "Cell Biology" with Tom Pollard and Bill Earnshaw and was President of the American Society of Cell Biology. Dr. Lippincott-Schwartz attended Swarthmore College, received her MS from Stanford University, and obtained her PhD in Biochemistry from Johns Hopkins University in 1986.

Jean and Joseph Sanger Lecture in Muscle Biology

James A. Spudich, PhD

Douglass M. and Nola Leishman Professor of Cardiovascular Disease, Stanford University

Dr. James Spudich, Douglass M. and Nola Leishman Professor of Cardiovascular Disease, is in the Department of Biochemistry at Stanford University School of Medicine. He received his B.S. in chemistry from the University of Illinois in 1963 and his Ph.D. in biochemistry from Stanford in 1968. He did postdoctoral work in genetics at Stanford and in structural biology at the MRC Laboratory in Cambridge, England. From 1971 to 1977, he was Assistant, Associate, and Full Professor in the Department of Biochemistry and Biophysics, University of California, San Francisco. In 1977, he was appointed Professor in the Department of Structural Biology at Stanford University. Dr. Spudich served as Chairman of the Department of Structural Biology from 1979-1984. Since 1992 he has been Professor in the Department of Biochemistry, where he served as Chairman from 1994-1998. From 1998 to 2002, he was Co-Founder and first Director of the Stanford Interdisciplinary Program in Bioengineering, Biomedicine and Biosciences called Bio-X. He is also an Adjunct Professor at the National Center for Biological Sciences, Tata Institute of Fundamental Research and InStem in Bangalore, India. Dr. Spudich is the Founder of four biotech companies: 1998 Cytokinetics, focused on treatments for diseases characterized by compromised muscle function like amyotrophic lateral sclerosis and heart failure, with several small molecule modulators in late stage clinical trials; 2012 MyoKardia, focused on developing targeted therapies for the treatment of rare genetically-based cardiovascular diseases such as hypertrophic and dilated cardiomyopathy, resulting in a $13.1B buyout by Bristol Myers Squibb and an FDA approved drug Camzyos (mavacamten); 2019 Kainomyx, focused on targeting cytoskeletal components of Plasmodium parasites for treatment of malaria; 2022 Cyntegron Therapeutics, focused on targeting cytoskeletal components for the treatment of cancers.

Dr. Spudich has given more than 50 named lectureships and keynote addresses, and has received many honors, including election to the National Academy of Sciences in 1991, and recipient of the Albert Lasker Basic Medical Research Award in 2012.

Over the last five decades, the Spudich laboratory studied the structure and function of the myosin family of molecular motors in vitro and in vivo, and they developed multiple new tools, including in vitro motility assays taken to the single molecule level using laser traps. That work led them to their current focus at Stanford on the human cardiac sarcomere and the molecular basis of hypertrophic and dilated cardiomyopathy. Spudich postulated in 2015 that a majority of hypertrophic cardiomyopathy mutations are likely to be shifting beta-cardiac myosin heads from a sequestered off-state to an active on-state for interaction with actin, resulting in the hyper-contractility seen clinically in HCM patients. This unifying hypothesis is different from earlier prevailing views, and this viewing an old disease in a new light has become the favored view in the field of the molecular basis of hypercontractility caused by HCM mutations. While maintaining his lab at Stanford, Spudich serves as CEO and President of both Kainomyx and Cyntegron Therapeutics.

Andrew P. Somlyo Honorary Lectures

Andrew P. Somlyo, MD
(1930 – 2004)

Professor of Physiology and Pathology and founding Director of the Pennsylvania Muscle Institute, Dr. Somlyo was a luminary in the field of smooth muscle physiology. His research (in collaboration with Dr. Avril Somlyo) played a key role in showing that actin-myosin interactions are responsible for force generation in smooth muscle. With colleagues at the University of Pennsylvania, Dr. Somlyo developed electron probe microanalysis to determine local ion concentrations in tissues at nanometer resolution. Additionally, his pioneering work in signaling revealed the mechanisms that regulate contraction of smooth muscle independently of the membrane potential – a process he termed pharmacomechanical coupling. Dr. Somlyo had a passion for science that is evident in the remarkable imprint that he left on the field of muscle physiology and on his students and colleagues. He was also a noted collector of Asian art. Dr. Somlyo left Penn Medicine in 1988 to chair the Department of Molecular Physiology and Biological Physics at the University of Virginia School of Medicine.

Jean and Joseph Sanger Lecture in Muscle Biology

Jean M. Sanger, PhD
Professor, Department of Cell and Developmental Biology
SUNY Upstate Medical University

Joseph W. Sanger, PhD
Professor, Department of Cell and Developmental Biology
SUNY Upstate Medical University

Drs. Jean and Joseph Sanger are pioneers in the development and use of fluorescently labeled proteins to examine the architecture and dynamics of a range of biological processes in developing and mature cells. As former members of the Department of Cell and Developmental Biology, they were founding members of the Pennsylvania Muscle Institute. The Sangers were among the first cell biologists to take advantage of probes to follow the assembly and changing localizations of cytoskeletal components in living cells. Their research led to impactful new discoveries about cell division, actin based bacterial infections, and assembly and maintenance of myofibrils in muscle cells. Importantly, the Sangers were the first scientists to visualize and quantify the kinetics of sarcomeric proteins

entering newly developing and mature myofibrils. In real time, they followed key components of the contractile machinery during myofibrillogenesis. The revolutionary models they formulated for how Z-bands, thick and thin filaments, and other sarcomeric components are templated during development are still the standards in the field. More recently, the Sangers were the first to determine the role of the ubiquitin–proteasome system in the progression of nascent myofibrils to maturity, and possible mechanisms for the off-target effects on hearts by chemotherapeutics. In addition to their scientific achievements, the Sangers have been leaders and role models in the Cell Biology community as educators, mentors to trainees and faculty, editors, reviewers, conference organizers, and administrators. Dr. Joseph Sanger served as interim chair of PSOM's Department of Cell and Developmental Biology. The Sangers left Penn in 2006 for SUNY Upstate Medical University, where Dr. Jean Sanger became Professor and Dr. Joseph Sanger became Professor and Chair of Cell and Developmental Biology. They were and are parts of the soul of the Pennsylvania Muscle Institute.

POSTER ABSTRACTS

1.

Model of how septin ring compartmentalization aids T-cell circumnavigation in extracellular matrices

Sami Alawadhi[1], David Rutkowski[1], Alexander Zhovmer[2], Erdem Tabdanov[3], Dimitrios Vavylonis[1]

1- Department of Physics, Lehigh University, Bethlehem PA
2- Center for Biologics Evaluation & Research, US Food and Drug Administration, Silver Spring, MD
3- Department of Pharmacology, Penn State University, Hershey-Hummelstown, PA

In order to efficiently migrate through complex environments, T cells adopt a variety of migration modes including amoeboid and mesenchymal motions. Their ability to push the nucleus through narrow passages is critical for their migration through the extracellular matrix. Zhovmer et al. recently discovered that T cells in collagen matrices move with the aid of septin rings, which form around the nucleus at locations where extracellular matrix obstacles create high negative cell curvature. The resulting septin/F-actin rings subdivide the volume of the cell into separate compartments, with potentially different microenvironments. We developed a 2D computational model to test how such compartmentalization aids cell motility. In the model, beads representing the plasma membrane and nucleus move according to forces of bending rigidity, tension, contraction, fluctuating protrusions, and excluded volume interactions from encountered obstacles. Cell and nuclear volume conservation are implemented to represent cytoskeleton- and organelle-mediated nuclear centering. We assume that septin ring formation leads to compartment boundaries at sites where obstacles enforce proximity between the cell membrane and nucleus. We show that formation of these boundaries leads to a nuclear piston mechanism that enhances motility at high obstacle density.

2.

Matrix stiffness suppresses growth but induces genomic instability and variation in cancer spheroids

Alişya A. Anlaş*[1], Brandon H. Hayes[2] Mai Wang[1], Markus Sprenger[2] and Dennis E. Discher[1,2]

University of Pennsylvania, [1]Department of Chemical and Biomolecular Engineering, [2]Department of Bioengineering

Genomic instability, the inability of a cell to pass on its genetic information accurately, is a hallmark of cancer. Aneuploidy, or an abnormal number of chromosomes, is observed in approximately 85% of solid tumors. During cancer progression, changes in the mechanical microenvironment can physically restrict cancer cells and induce errors in DNA replication or mitosis. Using chromosome reporter cell lines, we investigated whether a three-dimensional (3D) tumor microenvironment contributes to aneuploidy and found that increased matrix stiffness increases mitotic aberrations that may lead to chromosome missegregation. Our findings indicate that stiff microenvironments suppress mitosis, increase micronucleus formation, and enhance chromosome loss, thus highlighting a potential role for mechanical confinement in chromosome segregation. We also find that inhibiting myosin-II increases chromosome loss without affecting spheroid growth. The variance in chromosome loss across cancer spheroids increases per Luria-Delbruck's theory of heritable genetic change and is also consistent with the emergence of colonies with chromosome loss. Overall, our findings indicate that increased matrix stiffness increases heritable genomic instability and tumor heterogeneity -a deeper understanding of which could contribute to synergistic treatments for cancer.

3. ***Drosophila*** **Myo1C and Myo1D are high duty ratio motors with very high ADP affinity**

Faviolla A. Baez-Cruz and E. Michael Ostap

Unconventional class-I myosins (myosin-Is) are single-headed, actin-based motors that link membranes to the cytoskeleton and participate in a range of cellular functions. *Drosophila* has two myosin-I isoforms, myosin-1C (Myo1C) and myosin-1D (Myo1D), which have roles in establishing organismal chirality during development, likely through the interaction with E-cadherin and beta-catenin at sites of cell-cell adhesion. Remarkably, ectopic expression of either Myo1C or Myo1D in non-chiral organs (e.g., epithelium and trachea) result in generation of organs with distinct chirality, depending on the myosin-I isoform expressed. The activity of the myosin-I motor domains was shown to be crucial for this induced chirality, with the handedness of chirality depending on which motor domain was expressed. To better understand the motor properties of these myosins, we performed a detailed biochemical investigation of recombinant, full length Myo1C and Myo1D obtained by expression in Sf9 cells using baculovirus. The steady- state ATPase activities of the motors are actin-activated, with Myo1D having a Vmax 12.5- fold larger thanMyo1C, consistent with slower actin gliding speeds of Myo1C found via in vitro motility assay. ATP binding is fast and non-rate-limiting in both myosins. The maximum rate of actin-activated phosphate release (0.4 s^{-1}) from Myo1C is similar in magnitude to its ADP release rate (1 s^{-1}). However, the rate of ADP release from Myo1D (8 s^{-1}) is substantially slower than the rate of phosphate release (28 s^{-1}). Notably, the ADP affinities for both Myo1C (89 nM) and Myo1D (44 nM) are among the tightest measured for any myosins, and will likely result in substantial steady-state population of the force- bearing AM.ADP states under physiological nucleotide concentrations.

4. **Optical trapping assays to study force-dependent mobility of the diffusing microtubule associated proteins**

Fedor Balabin[1], Vladimir Demidov[1], Fazly Ataullakhanov[1,2], Ekaterina Grishchuk[1]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, Moscow, Russia

During cell division, the kinetochore-localized microtubule-associated proteins maintain mobile bonds with spindle microtubules. The key player in this process is the Ndc80 protein complex, which exhibits Brownian diffusion along microtubules in vitro. To gain understanding of its force-driven gliding on microtubules, specialized techniques are necessary to apply precise forces to individual diffusing proteins and their assemblies. We use a custom-built laser trapping instrument with a dual-trap three-bead setup. In this configuration, a microtubule is tethered to two trapped beads, forming a “dumbbell”, while the Ndc80 protein is attached to a stationary pedestal bead on a coverslip. This geometry minimizes vertical force component and imposes lateral Ndc80 translocation on microtubule, in contrast to experiments involving protein-coated beads that can roll along the microtubule under dragging forces. For our study on Ndc80 gliding, we utilize the ultrafast force-clamp (UFFC) technique, which applies forces from 2 to 15 pN through a feedback loop at 64 kHz. This allows us to detect and analyze gliding events lasting few milliseconds. Our findings reveal that the Ndc80 complex translocates via a mobile slip-bond when pulled toward the microtubule minus-end and a mobile catch-bond when pulled in the opposite direction. Dumbbell displacement in the UFFC is limited by the stationary laser detection range, thereby restricting our ability to study Ndc80 bound to the elongated CENP-T protein, which recruits Ndc80 to the kinetochore. To investigate gliding of the Ndc80-CENP-T complex and explore its force-sensitivity, we employ a dragging assay in which a CENP-T-coated pedestal is moved at a constant velocity along a suspended microtubule dumbbell using a piezo-stage. Our poster will provide an overview of these distinct force spectroscopy methodologies, discussing their advantages and limitations, and presenting our progress in studying Ndc80 recruitment to CENP-T.

5. **Cellular contractility controls extracellular matrix composition and function of developing hearts**

Susanna Belt and Karanvir Saini

Cardiovascular disease accounts for over a third of global mortality, with cardiac fibrosis alone affecting 6.2 million adults in the U.S. Caused by overaccumulation of collagen-rich extracellular matrix (ECM), current treatment options for progressive cardiac fibrosis focus on symptom management rather than restoration of "normal" levels of ECM proteins and tissue stiffness. The Rho kinases (ROCK) promote expression of pro-fibrotic genes via manipulation of acto-myosin based cellular contractility and are widely expressed by many cell-types, including fibroblasts. We examined the effects of cellular contractility on ECM collagen levels and cardiac function by isolating autonomously beating embryonic chick hearts composed of cardiomyocytes and fibroblasts, supported by single-cell RNA sequencing. We perturbed acto-myosin based cellular contractility using small-molecule inhibitors namely ROCK-2 (Belumosudil) and Blebbistatin (a pan myosin-II inhibitor), whereas a pan matrix metalloproteinase inhibitor (MMPi) and collagenases were applied to disrupt ECM protein levels. We applied wide-field time-lapse imaging simultaneously during drug perturbations to measure cardiac cellular-contractility and second harmonic imaging (SHG) to capture real-time kinetics of fibrillar cardiac collagen levels. We found that exposure of the heart tissues to ROCKi resulted in beating strain suppression similar to that of Blebbistatin-treated hearts. Given that ROCK-2 is primarily expressed by fibroblastic cells in the heart and cardiomyocytes express extremely low levels, the slower rate of beating strain inhibition during ROCKi treatments (~30 mins) compared to Blebbistatin-treated hearts (<15 mins). This likely results from cell-type specific distribution of ROCK-2 within the heart tissue while Blebbistatin targets several myosin types abundant in most cell-types present in the heart. Considering the propagation of contractile wave among sparse immature cardiomyocytes relies on robust cell-ECM interactions, ROCK-2 inhibition likely promotes ECM catabolism, disabling contractile wave propagation in the heart. Our results have potential to deepen the understanding of cell-based contractile forces on ECM in development, adaptation, and disease. The modulation of cellular forces during pathologies via targeting ROCK-pathway or other means might help target fibroblastic cells over other cell-types. Given that fibroblasts are primarily responsible for synthesis and degradation of a tissue ECM, such targeting of fibroblasts might help restore "normal" ECM and function of fibrotic hearts and other tissues.

6. **Identifying Molecular Heterogeneity in Endolysosomal Populations via Multiplexed DNA-PAINT Super-Resolution Microscopy**

Charles Bond, Siewert Hugelier, **Jiazheng Xing**, Elena M. Sorokina, Melike Lakadamyali

Cells harbor specialized membrane-bound organelles that regulate unique aspects of cellular homeostasis. Fluorescence microscopy is an indispensable tool for studying the dynamics and functions of these organelles. However, these studies typically rely on tagging a given organelle with a singular surface protein marker, which may not reflect an organelle's full identity. In addition, the frequent use of overexpressed protein markers can alter an organelle's identity. For example, LAMP1 and LAMP2 are canonical lysosomal markers widely employed in light microscopy studies. Mass spectrometry analyses confirm their high abundance on lysosomal membranes, alongside numerous other proteins. However, it remains unclear whether these proteins co-exist on the same lysosomal compartments or if there exists distinct subsets or even a continuous spectrum of lysosomes characterized by varying protein levels. To address these questions, we used DNA-PAINT super-resolution microscopy that combines high sensitivity and target specificity with high-order multiplexing, making it perfectly suited for systematic, quantitative studies of organelle heterogeneity. Using DNA-PAINT, we determined the intracellular organization of lysosomal compartments in HeLa and ARPE-19 cells by endogenously labeling them with combinations of multiple membrane protein markers including LAMP1, LAMP2, CD63, TMEM192, NPC1, and LAMTOR4. These experiments identified distinct sub-populations of

lysosomes enriched with subsets of these proteins, revealing their spatial distribution within the cell, as well as their relative proximity to other organelles. Most strikingly, only about 50% of LAMP1-positive lysosomes are marked by NPC1, suggesting only this subset is involved in cholesterol homeostasis. LAMTOR4, a component of the Ragulator complex, labels 80% of compartments, suggesting a broader population of lysosomes is involved in mTORC1-mediated metabolic homeostasis. Our findings unveil a previously overlooked heterogeneity among lysosomes, offering new avenues for exploring how these discrete sub-populations contribute to specific cellular functions. Additionally, our methodology opens the door for uncovering functional diversity among other types of organelles.

7.

Myosin with hypertrophic cardiomyopathy mutation M493I demonstrates altered crossbridge kinetics and force sensitivity despite normal working stroke

Robert C Cail, Donald A Winkelmann, Yale E Goldman and E Michael Ostap

Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant genetic disorder and a leading cause of sudden cardiac death. Hundreds of HCM-causing mutations have been identified in the gene MYH7, which encodes beta-cardiac myosin, but how these mutations change the phenotype of myosin is not known. Recent work in single-fiber and single-molecule studies of HCM-mutant myosins have demonstrated reduced force production, contradicting the long-standing model of enhanced contractility in HCM. Thus, mapping the mechanochemical effects of mutations on myosin function is essential to understanding disease etiology. Here, we use a combination of bulk-phase kinetics and single-molecule optical trap studies to determine the kinetics and mechanics of the severe HCM-causing mutation M493I. We find that M493I does not substantially affect the working stroke of single myosins (5.2 nanometers vs 5.1 nanometers for WT). However, M493I significantly decreases the rate of ADP release (13/s vs 70/s for WT), and slightly increases the rate of ATP binding (5.5/uM*s vs 4.5/uM*s for WT). Ensemble averages from M493I interactions with optically trapped actin dumbbells reveal similar kinetics for ADP release, but interestingly the kinetics of ATP binding do not change with increasing ATP concentration, evidence of a multi-step process leading to actomyosin detachment. Additionally, M493I myosin attaches strongly in the ADP-myosin state and produces enhanced force vs WT. Finally, while WT myosin's reattachment kinetics follow a double-exponential distribution, M493I's reattachment kinetics are single-exponential with only a fast phase, evidence of a disrupted super-relaxed myosin state. Together, these data demonstrate both kinetic and mechanical changes to beta-cardiac myosin bearing the mutation M493I, giving evidence of the underlying causes of septal restriction and hypertrophic phenotype in patients.

8.

MICU1, the mitochondrial Ca^{2+} uniporter gatekeeper, assists mitochondrial fusion dynamics by stabilizing mitochondrial fusion and preventing peri-mitochondrial actin filaments formation.

Cartes-Saavedra B[1]., Chakrabarti R[1]., Hasan P[1], Berezhnaya E[1]., Perocchi F[2]., Hajnóczky G[1].

[1]MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, PA;
[2]Institute of Neuronal Cell Biology, Technical University of Munich, Munich, Germany.

Introduction: Mitochondria are multifaceted organelles that provide energy for the main cellular processes. Mitochondrial Ca^{2+} (Ca^{2+}_m) signaling and mitochondrial fusion are central in organelle bioenergetics and quality control. Ca^{2+}_m uptake is mediated by the mitochondrial calcium uniporter complex (mtCU), a pore composed of a core (MCU), a scaffold (EMRE), and the mtCU gatekeepers MICU1 and MICU2. However, how mitochondrial fusion dynamics and Ca^{2+}_m signaling are connected remains elusive. In this work, we address how the loss of the MICU1 impairs mitochondrial fusion and its relationship with Ca^{2+}_m homeostasis. Material and Methods: To address our questions, we created acute and chronic MICU1 KO using a CRE-Lox recombination system. *MICU1$^{fl/fl}$* MEFs were infected

with a CRE or Null adenovirus for 72 hr to induce the recombination. To follow mitochondrial fusion dynamics, infected *MICU1$^{fl/fl}$* MEFs transfected with a mitochondrial-targeted photoswitchable proteins mtPAGFP /mtDsRed or mtDendra2. For $Ca^{2+}{}_m$ measurements, the cells were transfected with a mitochondrial matrix-Ca^{2+} sensor (mtRCaMP). Rhodamine Phalloidine was used to stain the Actin filaments. Results: MICU1 loss showed mitochondrial fusion inhibition in MEFs. Rescue of MICU1 restores the mitochondrial fusion activity to normal levels. Acute resce of MICU1 increases the fusion activity. Also, elevated resting $Ca^{2+}{}_m$ upon loss of MICU1 lead to a peri-mitochondrial actin polymerization around the mitochondria. Destabilization of the actin cytoskeleton by inhibiting the activity of the protein ARP2 using the drug CK666 showed restoration of mitochondrial fusion activity during acute or chronic loss of MICU1 in MEFs. However, the increase of fusion activity by CK666 in control cells was by decreasing the kiss-and-run and stabilizing the complete fusion events, while upon MICU1 loss was mostly by increasing the kiss-and-run fusion events. Discussion: Our results show that MICU1 loss leads to mitochondrial fusion impairment, with $Ca^{2+}{}_m$ dysregulation and peri-mitochondrial actin polymerization, an important player in fusion dynamics, as demonstrated in our experiments. Our results suggest that MICU1 has a dual role in fusion dynamics by stabilizing mitochondrial fusion and preventing the peri-mitochondrial actin formation.

9.

Study of ACTG2 mutations reveals different biochemical mechanisms underlying Visceral Myopathy

Rachel H. Ceron, Faviolla A. Báez-Cruz, Nicholas Palmer, Peter J. Carman, Malgorzata Boczkowska, E. Michael Ostap, Robert O. Heuckeroth, & Roberto Dominguez*

Visceral myopathy is a rare, debilitating disease in which dysfunctional smooth muscle in the bowel, bladder, and uterus hinders intestinal motility leading to abdominal distension, chronic constipation, and poor tolerance for enteral feeding. Patients with visceral myopathy often require multiple abdominal surgeries, intermittent bladder catheterization, intravenous nutrition, and even multi-visceral organ transplant to prolong life. The most common known cause of visceral myopathy are mutations in the gene encoding the gamma smooth muscle isoform of actin (ACTG2). Actin is an important component of the cytoskeleton and composes thin filaments in muscles for contraction with myosin motor proteins. Pathogenic ACTG2 variants in certain arginine residues (R40, R148, R178, and R257) are particularly common and have revealed a correlation between the mutated residue and disease severity. We recently developed a method for producing recombinant native-like human actin proteins for *in vitro* characterization and employed this novel purification scheme to study the biochemical mechanisms by which certain common *ACTG2* mutations (R40C, R148C, R179C, and R257C) cause disease. Surprisingly, all four studied variants had different effects on actin protein stability, interactions with actin binding proteins, ability to polymerize, and actin filament stability. The different biochemical deficits detected here may help explain the differences in disease severities for patients with different disease-causing *ACTG2* mutations.

10.

Intracellular enrichment of lipid droplets increases cytoskeletal polymerization and chromosome segregation errors

Geng-Yuan Chen[1], Lawrence J. Dooling[2], Megan Chung[1], Dennis E. Discher[2], Michael A. Lampson[1]

[1] Department of Biology, University of Pennsylvania
[2] Physical Sciences Oncology Ctr/Proj, University of Pennsylvania, PA, 19104

Cancer progression is highly associated with chromosome instability arising from mitotic errors, but the underlying mechanisms are difficult to explain simply by mutations in oncogenes or tumor suppressor genes. Physical constraints, such as extracellular constraints by a stiff tumor microenvironment, can also cause mitotic errors independent of genetic defects. Hepatic cells provide a natural system to study the impacts of physical constraints on mitosis because they deposit intracellular lipid droplets and

	extracellular matrices during cancer progression. We show that droplet enrichment reduces cytoplasmic volume in mitosis, suggesting increased intracellular protein concentrations that favor cytoskeletal polymerization. Consistently, droplet enrichment increases actin polymerization and cortical tension by increasing total actin concentration, suggesting increased cortical actomyosin contractility. Droplet enrichment also increases total tubulin concentration, spindle microtubule polymerization, and chromosome segregation errors, suggesting impaired regulation of kinetochore-microtubule attachments by increasing microtubule polymers. We propose that cytoskeletal polymerization by droplet enrichment confers two characteristics of cancer cell proliferation. First, increased cortical actomyosin contractility can help cells push against the stiff microenvironment for viable mitosis. Second, increased microtubule polymers generate chromosome instability, independent of genetic defects.
11.	**Delivery and imaging of functionalized DNA origami in cells** **P.L. Colosi**, Elena M. Sorokina, Golbarg M. Roozbahani, Wolfgang Pfeifer, Atila Oravecz, Laszlo Tora, Michael Poirier, Melike Lakadamyali, Carlos Castro Human cells package over two meters of genetic information into the microscopic space of the nucleus in the form of chromatin (a complex between DNA and histone proteins). This packaging or "folding" of the genome is critical for the regulation of gene expression, maintenance of genomic stability, and defining cell fate during development. Change to the spatial organization of chromatin is implicated in a host of neurological disorders as well as in aging and cancer. Remodeling of chromatin via post-translational modifications allows for epigenetic regulation of transcription in healthy and diseased cells. Thus, tools to engineer chromatin epigenetics in a controlled manner are crucial to study the cause-consequence relationship between epigenetics, chromatin organization and gene expression. Previous approaches to chromatin engineering have significant limitations, namely the inability to perform multiple functions (visualize, modify, or detect downstream products) simultaneously. Our work seeks to develop and apply DNA origami nanodevices (DOs) as multi-functional platforms to probe and engineer chromatin epigenetics. We propose to leverage the versatility of DO and its potential as a platform for multiple functional elements to visualize, modify, and sense the transcriptional output of genomic regions in mammalian cell nuclei. We have taken an interdisciplinary approach to develop and test four different DNA origami structures (26-helix bundle, 14-helix bundle, 13-helix bundle, and 8-helix bundle) functionalized with fluorophores as well as an anti-RNA Polymerase II (Pol2) antibody. We show that electroporation enables delivery of these structures into cells and further, 8-helix bundle structures functionalized with RNA Pol2 antibodies are successfully piggybacked into the nucleus. Super-resolution imaging further demonstrates the delivery of single DNA origami structures into the cell nucleus. These results open the door for DNA origami as a multifunctional platform for targeting, modifying, and visualizing the genome.
12.	**Ultrastructure of human brain tissue vitrified directly from autopsy revealed by cryo-ET with cryo-plasma FIB milling** **Benjamin C. Creekmore**, Kathryn Kixmoeller, Ben E. Black, Edward B. Lee, Yi-Wei Chang Ultrastructure of human brain tissue has traditionally been examined using electron microscopy (EM) following fixation, staining, and sectioning, which limit resolution and introduce artifacts. Alternatively, cryo-electron tomography (cryo-ET) allows higher resolution imaging of unfixed cellular samples while preserving architecture, but it requires samples to be vitreous and thin enough for transmission EM. Due to these requirements, cryo-ET has yet to be employed to investigate unfixed, never previously frozen human brain tissue. Here we present a method for generating lamellae in human brain tissue obtained at time of autopsy that can be imaged via cryo-ET. We vitrify the tissue via plunge-freezing and use xenon plasma focused ion beam (FIB) milling to generate lamellae directly on-grid at variable depth inside the tissue. Lamellae generated in Alzheimer's disease brain tissue reveal intact subcellular structures including components of autophagy and potential pathologic tau fibrils. Furthermore, we reveal intact

compact myelin and functional cytoplasmic expansions including insight into how myelin basic protein forms compact myelin. This method is designed to be broadly accessible for different tissue systems to allow for visualization of a variety of delicate subcellular structures in a more native context.

13. **Cooperative phagocytosis by macrophages overcomes rapid growth and cohesiveness of solid tumors**

Lawrence J. Dooling, Ph.D., Jason C. Andrechak, Ph.D., Brandon H. Hayes, Tristan Marchena, Nicholas Ontko, Dennis E. Discher, Ph.D

Macrophages are abundant in solid tumors and typically associate with poor prognosis, but macrophage aggregates have also been reported as beneficial in some tumor types even though dispersed macrophages would have greater contact with target cancer cells. Here, by maximizing phagocytic activity through IgG opsonization of tumor cells and disruption of the CD47-SIRPα macrophage checkpoint, we discover cooperative phagocytosis by low entropy clusters in rapidly growing engineered 'immuno-tumoroids'. The results fit a phenomenological model of proliferation versus engulfment while rheological measurements and molecular perturbations provide a basis for understanding macrophage aggregation and phagocytic disruption of a cohesive forces in soft cellular phases. Combining complete disruption of CD47-SIRPα with an otherwise ineffective opsonizing monoclonal antibody *in vivo* suppresses tumor growth and durably protects some mice from re-challenge and metastasis. Clusters of phagocytic macrophages in treated tumors and the relative scarceness of partial responses compared to complete and non-responses are consistent with the cooperative, aggregating macrophages observed in tumoroids. Systemic delivery of engineered macrophages increases long-term survival (≥60% mice) and potentially overcomes challenges for solid tumor therapies that include limited permeation of antibodies. Induced anti-cancer IgG in convalescent sera prove both tumor-specific and multi-epitope, which possibly contribute to a phagocytic feedback *in vivo* but certainly drive macrophage clustering *in vitro*. Given that solid tumors remain challenging for immunotherapies, durable anti-tumor responses here illustrate unexpected advantages in maximizing macrophage density and net phagocytic activity.

14. **FMRP-associated protein synthesis locally determines mitochondrial organization in neurons**

Adam R. Fenton[1-4], Ruchao Peng[5], Charles Bond[2,3], Siewert Hugelier[2], Melike Lakadamyali[2-4], Yi-Wei Chang[4-5], Erika L. F. Holzbaur[2-4], Thomas A. Jongens[1,3]

[1]Department of Genetics, University of Pennsylvania Perelman School of Medicine
[2]Department of Physiology, University of Pennsylvania Perelman School of Medicine
[3]Cell and Molecular Biology Graduate Group, University of Pennsylvania Perelman School of Medicine
[4]Pennsylvania Muscle Institute, University of Pennsylvania Perelman School of Medicine
[5]Department of Biochemistry and Biophysics, University of Pennsylvania Perelman School of Medicine

Mitochondrial health and function are maintained in neurons by network remodeling and local translation, which allow for rapid responses to changing cellular demands. Yet, it is not understood how neurons orchestrate the timing and positioning of translation or how local translation is coupled with mitochondrial dynamics to maintain network integrity. The Fragile X Messenger Ribonucleoprotein Protein (FMRP) is a critical regulator of translation in neurons whose absence causes Fragile X Syndrome, a severe neurodevelopmental disorder. Loss of FMRP disrupts mitochondrial health in neurons, resulting in a fragmented network with impaired metabolic function. However, the mechanism by which FMRP supports mitochondrial homeostasis in neurons is not known. Here, we use DNA-PAINT super-resolution microscopy and live-cell confocal microscopy to demonstrate close contacts between FMRP granules and mitochondria in mammalian neurons. FMRP preferentially clusters at sub-

	organellar regions of mitochondria: the ends and the midzone. End contacts are dynamic and allow for long-distance co-transport of FMRP with mitochondria. Midzone-associated FMRP marks sites of mitochondrial fission, where the FMRP granule stays associated with the mitochondrial end following fission. Endosomes are known to associate with sites of mitochondrial fission and to deliver RNA granules to mitochondria in neurons. We demonstrate that endosomes contact FMRP granules and contribute to their positioning at mitochondrial ends and fission sites. Using a combination of live-imaging, RNA *in situ* hybridization, immunocytochemistry, and cryo-electron tomography, we demonstrate that mitochondria-associated FMRP granules are ribosome-rich sites of protein synthesis, which contain mRNAs for nuclear-encoded proteins that drive mitochondrial function and dynamics. Further, mitochondrial fission at FMRP granules is dependent on protein synthesis and facilitated by local translation of Mitochondrial Fission Factor within these granules. These findings reveal a role for FMRP in the control of mitochondrial fission and suggest that FMRP granules serve as platforms to selectively regulate the dynamics of individual mitochondria in distal parts of neurons.
15.	**HFpEF animal models display differences in myofibril mechanics** **Axel Fenwick**[1], Vivek Jani[1,2], David Lefer[3], Thomas E. Sharp[4], Traci T. Goodchild[3], Kyle LaPenna[4], Joseph A. Hill[5], David Kass[1,2], Anthony Cammarato[1] Diastolic dysfunction is a hallmark of heart failure with preserved ejection fraction (HFpEF). However, it is unknown whether this organ-level deficiency in relaxation persists to the level of myofibrils, the contractile organelles within cells. To assess potential differences in myofibrillar force and relaxation kinetics, we performed mechanical analysis of individual myofibrils isolated from three HFpEF animal models: the Göttingen minipig, the ZSF1-obese rat, and mice exposed to a high-fat diet and the constitutive nitric oxide synthase inhibitor L-NAME. Myofibrils from HFpEF minipigs produced less maximal active tension compared to controls, but without differences in resting tension. ZSF1-obese rats and HFD+L-NAME mice, however, produced similar myofibrillar active and resting tension vs. controls. With respect to relaxation kinetics, we did not resolve differences in the duration or rate of the initial slow linear or subsequent fast exponential relaxation phases in the minipig model. However, both rodent models displayed a slower rate and prolonged duration of the initial linear relaxation phase, with the ZSF1 model also exhibiting slowing of the exponential relaxation phase. Together, these data reveal depressed systolic myofibrillar function only in the pig model. Whereas all three HFpEF models displayed signs of organ-level diastolic dysfunction, we show that this dysfunction is reflected at the subcellular level, through impaired myofibril relaxation, in only the ZSF1-obese rat and the HFD+L-NAME mouse. We have also begun collecting human myofibril data from HFpEF patient biopsies. Preliminary data of myofibrils from a specific subphenotype revealed a prolonged linear relaxation phase, indicating excessive actomyosin interactions following calcium removal. Ongoing studies on additional patient samples aim to determine which models best replicate different subphenotypes and to further understand the role of myofibril relaxation in HFpEF.
16.	**Biochemical and structural investigation of Arp2/3 complex regulation by mammalian coronin-7** **Fred Erick Fregoso**, Malgorzata Boczkowska, Grzegorz Rebowski, and Roberto Dominguez The ability for cells to rapidly reorganize cytoskeletal components in response stimuli is critical for a wide variety of important cellular processes. Such components are actin filaments (F-actin) and the Arp2/3 complex. The Arp2/3 complex generates branched actin networks by binding the side of an existing mother filament and nucleating a daughter, resulting in its structural incorporation at the branch junction. The lifetime of branched networks is regulated by proteins that stabilize/destabilize junction integrity, like cortactin and coronin respectively. Despite being one of the first Arp2/3 complex binding partners discovered, biochemical and structural insights of the coronin family of proteins remain extremely lacking. The yeast coronin system, which contains only one coronin gene, had been the

primary experimental model in the field. However, there is little to no studies on the seven mammalian coronin isoforms. We focus here on coronin-7 that differs most from other coronins in that it contains a C-terminal Central-Acidic (CA) domains like those of N-WASP-family proteins that activate Arp2/3 complex instead of a oligomerization domain. It is currently not known whether coronin-7 directly interacts with Arp2/3 complex or the cellular consequence that results when disrupting this interaction. Here, we show here that coronin-7 binds Arp2/3 complex through its CA region and competes with N-WASP for the same binding site on the complex. We also show that the full length coronin-7 binds F-actin. We additionally have evidence that suggests coronin-7 autoinhibition where the CA domain is sequestered away from solution. Lastly, knockdown of coronin-7 in MCF10A cells results in compromised cell motility. Our preliminary studies indicate that coronin-7 may exists in an autoinhibited state where upon its release by upstream signaling cascades, exposes its C-terminal CA domain and binds Arp2/3 complex to main proper cell motility.

17.

TMEM65 regulates NCLX-dependent mitochondrial calcium efflux

Joanne F. Garbincius[a], Oniel Salik[a], Henry M. Cohen[a], Carmen Choya-Foces [a,b], Adam S. Mangold[a], Angelina D. Makhoul[a], Anna E. Schmidt[a], Dima Y. Khalil[a], Joshua J. Doolittle[a], Anya S. Wilkinson[a], Emma K. Murray[a], Michael P. Lazaropoulos[a], Alycia N. Hildebrand[a], Dhanendra Tomar[a,c], and John W. Elrod[a]

[a]Aging + Cardiovascular Discovery Center, Department of Cardiovascular Sciences, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA
[b] Unidad de Investigación, Hospital Universitario Santa Cristina, Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, Spain
[c]Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

The balance between mitochondrial calcium ($_mCa^{2+}$) uptake and efflux regulates ATP production, but if perturbed causes energy starvation or $_mCa^{2+}$ overload and cell death. The mitochondrial sodium-calcium exchanger, NCLX, is a critical route of $_mCa^{2+}$ efflux in excitable tissues, such as the heart and brain, and animal models support NCLX as a promising therapeutic target to limit pathogenic $_mCa^{2+}$ overload. However, the mechanisms that regulate NCLX activity remain largely unknown. We used proximity biotinylation proteomic screening to identify the NCLX interactome and define novel regulators of NCLX function. Here, we discover the mitochondrial inner membrane protein, TMEM65, as an NCLX-proximal protein that potently enhances sodium (Na^+)-dependent $_mCa^{2+}$ efflux. Mechanistically, acute pharmacologic NCLX inhibition or genetic deletion of NCLX ablates the TMEM65-dependent increase in $_mCa^{2+}$ efflux. Further, loss-of-function studies show that TMEM65 is required for Na^+-dependent $_mCa^{2+}$ efflux. Co-fractionation and *in silico* structural modeling of TMEM65 and NCLX suggest these two proteins exist in a common macromolecular complex in which TMEM65 directly stimulates NCLX function. In line with these findings, knockdown of *Tmem65* in mice promotes $_mCa^{2+}$ overload in the heart and skeletal muscle and impairs both cardiac and neuromuscular function. We further demonstrate that *TMEM65* deletion causes excessive mitochondrial permeability transition, whereas TMEM65 overexpression protects against necrotic cell death during cellular Ca^{2+} stress. Collectively, our results show that loss of TMEM65 function in excitable tissue disrupts NCLX-dependent $_mCa^{2+}$ efflux, causing pathogenic $_mCa^{2+}$ overload, cell death and organ-level dysfunction, and that gain of TMEM65 function mitigates these effects. These findings demonstrate the essential role of TMEM65 in regulating NCLX-dependent $_mCa^{2+}$ efflux and suggest modulation of TMEM65 as a novel strategy for the therapeutic control of $_mCa^{2+}$ homeostasis.

18.

Temperature-dependent fret reveals uncoupling of the super-relaxed state and interacting heads motif

Jinghua Ge[1], Kevin E. Namitz[2], Skylar M.L. Bodt[1], Ianna S. Debrunner[3], Sivaraj Sivaramakrishnan[3], Neela Yennawar[2] and Christopher M. Yengo[1]

[1]Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA, USA
[2]Huck Institute of Life Science, Pennsylvania State University, University Park, PA, USA
[3]Genetics, Cell and Developmental Biology, University of Minnesota, Minneapolis, MN, USA

The structural basis of the slow ATP turnover in the super-relaxed (SRX) state of cardiac myosin is currently unclear. There is evidence that a conformation with the heads folded back on the tail and interacting with each other called the interacting heads motif (IHM) can stabilize the SRX biochemical state. We designed a FRET biosensor of the IHM structural state, by utilizing a beta- cardiac myosin heavy meromyosin construct with a C-terminal GFP tag (M2β HMM). We observed FRET between the C-terminal GFP tag and Cy3ATP in the active site, that we predicted would only occur with the heads folded back in the IHM. In this study, we examined the temperature-dependence of the SRX and IHM to examine how well they are correlated. In WT M2β HMM, we observed an decrease in the SRX state as a function of temperature with no change in the IHM FRET (10-25°C). In E525K M2β HMM, a mutant we previously reported stabilizes the SRX state and IHM, we found that temperature did not alter the SRX state and IHM FRET measurements. To further investigate the structural properties of the M2β HMM construct we performed analytical ultracentrifugation at varying salt concentrations (KCl). We found that altering KCl concentration altered the sedimentation coefficient and frictional ratio, consistent with an increase in the more compact IHM structure at lower KCl concentrations. In addition, the E525K mutation displayed a decreased frictional ratio compared to WT at all KCl concentrations measured, consistent with an increase in the stability of the IHM. Overall, this study demonstrates that the SRX state and IHM can be uncoupled, which suggests the SRX state may populate more than one structural state.

19.

Tissue Engineered Motor Units Featuring Spinal Motor Neurons and Dorsal Root Ganglia Sensory Neurons Innervating Myofiber Bundles

M.C. Hilman[1,2], S. Das[2, 3], D. K. Cullen[1, 2, 3]

[1] Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA
[2] Center for Neurotrauma, Neurodegeneration & Restoration, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA, USA
[3] Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Introduction: Tissue engineering strategies are being pursued to treat severe neuromuscular injuries by mimicking aspects of native myofascicular architecture and providing topographical guidance to regenerating motor units. A motor unit is the functional unit of muscle contraction and consists of a motor neuron and the population of skeletal muscle fibers innervated by the neuron's axon terminals. Our group previously developed pre-innervated tissue engineered muscle sheets comprised of planar myofibers in co-culture with spinal motor neurons grown on a nanofiber scaffold. This co-culture system showed that the presence of motor neurons facilitated myofiber maturation *in vitro* and promoted a pro-regenerative microenvironment following implant in a rat model of neuromuscular injury. While promising, the planar nature of this pre-innervated muscle did not reflect the three-dimensional (3D) bundled architecture of myofibers *in vivo*. In response, we have developed Tissue Engineered Motor Units (TEMUs) comprised of centimeter-scale aligned bundles of myofibers encased within a 3D collagen extracellular matrix (ECM) hydrogel and innervated by axons projecting from a discrete population(s) of spinal motor neurons and dorsal root ganglion (DRG) sensory neurons.
Methods: Our system facilitates the self-assembly of myoblasts into aligned 3D bundles of myofibers measuring millimeters in diameter and spanning centimeters in length. Using this platform, we investigated the combined effects of channel topography and innervation on myocyte maturation and contraction. We dispensed a homogenous solution comprising ECM + C2C12 mouse myoblasts into a 3D-printed channel scaffolding and placed spinal motor neuron aggregates on either end of the channel. A separate iteration included a sensory neuron population in the form of DRG, replacing one of the

motor neuron aggregates. After variable time – 7 or 21 days in culture –myofiber bundle thickness, fusion index, axon ingrowth, and neuromuscular junction formation were assessed through immunocytochemistry and confocal microscopy.

Results: Skeletal myocytes were successfully co-cultured with motor and sensory neurons in a columnar architecture. Cross-sectional histological analysis showed that TEMUs exhibited fascicle-like organization, exhibiting myofiber bundles and axons projecting along and within the ECM (**Fig. 1**). The tissue engineered constructs were formed at variable lengths (1, 2, 4, 8cm) with uniform myocyte cell density, neuron cell bodies at the extremes, and axons projecting along the length. Ongoing studies are using the TEMU platform to further establish the critical role of motor and sensory axon innervation on the development, maturation, functionality, and regenerative potential of engineered 3D myofiber bundles.

Conclusion: To overcome the inherent limitations of engineering complex multicellular muscle tissue, there is a critical need for an approach that can simultaneously (1) recapitulate the fascicular architecture and cellular niche of skeletal muscle, and (2) provide controlled axonal inputs from multiple neuronal populations to recreate motor units. TEMUs comprised of centimeter-scale aligned bundles of myocytes with axonal innervation from discrete motor and/or sensory neuron populations may provide such a platform. TEMUs may aid in addressing key challenges in the biofabrication of tissue engineering muscle as an *in vitro* test platform or as implantable constructs to facilitate muscle replacement after severe trauma.

20.

ECLiPSE: a versatile approach for structural and morphological analysis of 2D and 3D super-resolution fluorescence microscopy data

Siewert Hugelier[1], Hannah Kim[1], Melina Theoni Gyparaki[1], Charles Bond[1], Qing Tang[1], Adriana Naomi Santiago-Ruiz[1], Sílvia Porta[2], Melike Lakadamyali[1, 3]

[1]Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Center for Neurodegenerative Disease Research, University of Pennsylvania, Philadelphia, PA 19104, USA
[3]Epigenetics Institute, University of Pennsylvania, Philadelphia, PA 19104, USA

The recent advancements in super-resolution microscopy have revolutionized our ability to visualize the intricate morphological features of sub-cellular compartments and organelles at nanoscale spatial resolution. Super-resolution microscopy allows capturing subtle changes in the morphology and structure of these subcellular components, which can indicate changes to cellular homeostasis. For example, aggregation of proteins into morphologically diverse insoluble inclusions is a hallmark of several neurodegenerative diseases. However, the development of tools to accurately classify individual sub- cellular structures, organelles or protein aggregates into distinct categories based on shape and morphology has not kept pace with advancements in super-resolution microscopy.To bridge this gap, we developed a pipeline, which we called Enhanced Classification of Localized Pointclouds by Shape Extraction (ECLiPSE). It leverages a wide range of comprehensive shape descriptors encompassing geometric, boundary, skeleton and other properties, the majority of which are directly extracted from the 2D as well as 3D localizations to accurately characterize the morphology of individual structures. We also included automatic feature selection, which uses a priori information to select the most informative descriptors in distinguishing among different classes, while discarding those that are less informative. Exceptionally high classification accuracies nearing 100% were obtained on five distinct sub-cellular structures including organelles, cytoskeletal filaments and protein aggregates. Moreover, we demonstrate the versatility of ECLiPSE by applying it to two novel biological applications: quantifying the clearance of Tau protein aggregates, a critical marker for neurodegenerative diseases, and differentiating between two distinct strains of TAR DNA-binding protein 43 proteinopathy, each exhibiting unique seeding and spreading properties. To sum up, ECLiPSE has proven to be highly effective, and we anticipate that this versatile approach will significantly enhance the way we study cellular structures in 2D and 3D across various biological contexts.

21.

Interplay between AKT and AMPK signaling in skeletal muscle in the regulation of glucose homeostasis

Natasha Jaiswal[1], Matthew Gavin[1], Louise Lantier[2], David H. Wasserman[2] and Paul M. Titchenell[1,3]

[1]Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine, University of Pennsylvania
[2]Vanderbilt Mouse Metabolic Phenotyping Center, Nashville, TN and
[3]Department of Physiology, Perelman School of Medicine at the University of Pennsylvania

Insulin resistance is considered to be the principal factor underlying several metabolic diseases including type II diabetes mellitus. Since, skeletal muscle is the predominant site of insulin- mediated glucose uptake in the postprandial state, a reduction in the insulin signaling pathway of diabetic skeletal muscle is widely considered to be the primary cause of postprandial hyperglycemia. The serine/threonine kinase AKT is a central regulator of insulin action and a decrease in AKT activity is observed in muscle from insulin-resistant mice and humans. This has understandably led to the dogma that impaired AKT activity in skeletal muscle causes insulin resistance and defects in glucose homeostasis and muscle function. To test the direct requirement of skeletal muscle AKT signaling on systemic glucose metabolism and muscle physiology, we generated several mouse models of skeletal muscle AKT deficiency. Unexpectedly, mice lacking AKT2 alone, exhibited normal insulin signaling, insulin sensitivity, and muscle mass despite a dramatic reduction in phosphorylated muscle AKT. In contrast, deletion of both muscle AKT isoforms (M-AKTDKO) resulted in a complete loss of AKT-mediated insulin signaling. Surprisingly, despite the lack of AKT activity, M-AKTDKO mice were insulin sensitive and displayed normal rates of glucose uptake in response to insulin. These results demonstrate that AKT is not an obligate intermediate for insulin-stimulated glucose uptake in all conditions and suggests the existence of additional insulin-dependent, AKT-independent signaling pathways for the regulation of glucose homeostasis. An unbiased phosphoproteomics study reveals activation of PDPK-1, a PI3K substrate, uniquely in M-AKTDKO muscles in response to insulin. This was associated with the significant inhibition in phosphorylation of IRS2 at Ser303 and Ser577 (recently identified AKT-dependent phosphosites on IRS2 that limit PI3K signaling) and activation of the AMPK pathway in M-AKTDKO muscles in response to insulin. Intriguingly, combined inhibition and both AKT and AMPK pathway were required and sufficient to cause skeletal insulin resistance and alters glucose homeostasis. Collectively, these data define the new role of AKT in controlling insulin mediated AMPK pathway to control glucose uptake via negative feedback inhibition of insulin signaling by negatively regulating insulin signaling.

22.

Moesin influence integrin-dependent T cell migration

Marie Juzans, and Janis K. Burkhardt

Dept. of Pathology and Laboratory Medicine, Perelman School of Medicine of the University of Pennsylvania – Children's Hospital of Philadelphia Research Institute

The cell cortex is a thin meshwork of actin filaments and associated actin-binding proteins that lies just beneath the plasma membrane. Detachment of the cortex from the membrane permits protrusion formation, while stress gradients result in local contractions. Therefore, the cortex regulates lamellipodia formation and uropod retraction during cell migration. In T cells, moesin is a key regulator of cortex-membrane attachment, and expression of moesin mutants perturbs T cell motility. However, little is known about which aspects of T cell motility depend on moesin function, or how this is regulated by specific signaling pathways. I have identified a regulatory role for moesin during integrin-dependent T cell migration. Using CD4+ T cells from moesin knockout mice, I find that loss of moesin expression leads to enhanced adhesion to ICAM-1 coated surfaces. This is linked to increased cell deformability, increased integrin clustering, and faster polarization. Interestingly, moesin KO cells are less adhesive than WT cells on VCAM-1 coated surfaces, pointing to a role for moesin in controlling integrin-specific cellular responses. Taken together, these results indicate that efficient T cell migration is regulated by

	moesin at several levels. Going forward, it will be important to understand how distinct environmental cues differentially modulate moesin function to control cortical actin remodeling, and how this process results in specific migratory behaviors.
23.	**Investigating the chromatin state of *in vitro* chondrocyte dedifferentiation with super-resolution microscopy** **Hannah Kim**[1,2], Ellen Zhang[3], Su Chin Heo[3], Robert L. Mauck[3], and Melike Lakadamyali[2] Departments of [1]Biochemistry & Biophysics, [2]Physiology and, [3]Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 Chondrocytes form articular cartilage, the tissue that protects joints, by depositing key proteins and other molecules in their extracellular matrix (ECM). When cultured *in vitro*, some chondrocytes maintain their naïve phenotype while others experience dedifferentiation, altering morphology and reducing ECM production. This is especially an issue in tissue-engineering therapies designed to reimplant patient-derived chondrocytes expanded *in vitro*. In eukaryotic nuclei, chromatin organization regulates transcription machinery's access to DNA. Hence, chromatin state is heavily related to a cell's phenotype. Although chondrocyte dedifferentiation has been previously examined from a transcriptional and cellular signaling perspective, the link between global chromatin organization and chondrocyte phenotype has been relatively overlooked. We hypothesize that global shifts in chromatin reflect the altered cell state of dedifferentiating chondrocytes and that hindering these changes in chromatin may in turn reduce or reverse the progression of *in vitro* dedifferentiation. Utilizing STochastic Optical Reconstruction Microscopy (STORM) of histone proteins to quantify the spatial organization of chromatin beyond the diffraction limit of light, this study aims to explore the relationship between the ECM production of individual cells and their corresponding chromatin state. Preliminary results indicate that chromatin clusters experience an overall increase in compaction with increasing passage and in the absence of TGF-β3 treatment, which is known to promote the naïve chondrocyte phenotype.
24.	**Hsp104 is a stochastic processive translocase that can travel in both directions** **JiaBei Lin***, Yale E. Goldman#*, and James Shorter* *Department of Biochemistry & Biophysics, Perelman School of Medicine at The University of Pennsylvania. # Department of Physiology, Perelman School of Medicine at The University of Pennsylvania Hsp104, a hexameric AAA+ (ATPases associated with diverse cellular activities) ATPase, plays a critical role in yeast stress tolerance by restoring damaged and aggregated proteins to their soluble state. Like other AAA+ motors such as ClpA, ClpB, and ClpX, cyo-electron microscopy (Cryo-EM) studies have revealed non-symmetric, staircase-like hexameric structures that suggest a sequential right-handed/2-residue step translocation model. However, this model has not been confirmed experimentally. In this study, we have for the first time, investigated the conformational changes and translocation kinetics of Hsp104 in solution using single-molecule Total Internal Reflection Fluorescence (TIRF) microscopy. Two different model substrates, casein and the yeast Sup35 prion domain, were employed. Our findings show that Hsp104 functions as a stochastic motor, and its translocation processivity is substrate-dependent. There may be instances of free subunit exchange of Hsp104 hexamer while translocating on the substrate. Additionally, we verified the existence of an asymmetric seam in Hsp104 at two distinct distances in addition to the closed interface. Interestingly, one of these distances aligns with the structure of extended states of Hsp104. Furthermore, Hsp104 can move in both directions, toward the N-terminus of the substrate or toward the C-terminus, and it can reverse direction. This plasticity in motion likely enables Hsp104 to disentangle complex aggregated structures effectively.

25.

The timing of *Bmal1* deletion differentially impacts skeletal muscle morphology, function, and features of aging

Ronan Lordan[1], Sarah L. Teegarden[1], Sarah C. McLoughlin[1], Sean Kelch[1], Nicholas Lahens, Georgios Paschos[1], and Garret A. FitzGerald[1]

[1]Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Circadian clocks regulate biological functions via rhythmic gene expression orchestrated by *Bmal1 (Arntl)* and *Clock*. Embryonic *Bmal1*$^{-/-}$ knockout mice (eKO) have a shortened lifespan and exhibit a phenotype of accelerated ageing including an age-dependent reduction of muscle mass and altered muscle physiology [1,2]. Despite an identical impact on behavioral rhythms, lifespan, muscle mass and function are normal in postnatal *Bmal1*$^{-/-}$ mice (iKO). Considering exercise may attenuate the effects of sarcopenia in humans [3], in this study we examine exercise tolerance and aging in eKO and iKO mice. Both models were subjected to endurance exercise training on a treadmill for 3 times/week at ZT 0-4 for 50 min (17 m/min) for 8 weeks. Post training, an exercise tolerance test indicated that sedentary eKO mice show exercise deficits versus wild type (WT) controls that can be rescued by endurance training; iKO mice, by contrast, do not exhibit a deficit in exercise tolerance. Sedentary cKO mice had increased fibrosis and skeletal muscle central nuclei (indicative of regeneration) than WT, and these features increased upon exercise. These changes were less marked in iKOs. Analyses of plasma, gastrocnemius, and heart acylcarnitines indicate that eKO mice have a deficit of medium and long-chain acylcarnitines that are recued to WT baseline levels in response to exercise. Deficits in the iKO at baseline are not as severe as eKO mice but do respond similarly to exercise. These data and microscopy of the soleus muscle implicate mitochondrial dysfunction in the exercise phenotypes and anatomical deficits of the cKO mice. In conclusion, the timing of *Bmal1* deletion differentially impacts exercise tolerance. *Bmal1* absence during development results in a phenotype of premature aging in skeletal muscle that is attenuated by endurance exercise. Postnatal loss of *Bmal1* has minimal effects on muscle morphology and function.

26.

Reconstitution of kinetochore complexes using genetically engineered multimers

Aleksandr Maiorov, Ekaterina Tarasovetc, Ekaterina L. Grishchuk

Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Prior studies in cells and in vitro revealed features of human kinetochore architecture and assembly pathways. Scaffolding CENP-C protein, that present in multiple copies at centromere, plays a crucial role in recruiting outer kinetochore components. Mis12 complex binds N-terminal end of CENP-C, then the Ndc80 complex binds Mis12 through Spc24/Spc25 domains. These studies also unveiled the role of Aurora B kinase phosphorylation of the Mis12 complex, which enhances the binding affinity of the Mis12 complex to CENP-C. Past reconstructions in vitro used Mis12 and CENP-C proteins at concentrations exceeding physiological levels. Moreover, CENP-C was used in a soluble form, while in cells the CENP-C is clustered at the centromere. Our recent work using other protein CENP-T showed that binding interactions may differ significantly between the proteins in the soluble vs. clustered form. To overcome these limitations, we developed an approach in which the N-terminal ends of CENP-C are clustered using genetically engineered multimers (GEMs). This technique enables control of system components, as well as accurate measurement of the Mis12 complex binding to clustered CENP-C. We used real-time TIRF microscopy to track interactions between CENP-C-eGFP clusters and the recombinant Alexa647-labeled Mis12/KNL1 complex. At 100 nM of Mis12/KNL1, its binding saturation to CENP-C clusters was observed within few minutes, with a 1:1 ratio of Mis12/KNL1 to CENP-C molecules, as expected. Subsequent addition of 200 nM of soluble Ndc80, which is similar to the estimated cellular concentration, led to recruitment of ~ 0.5 Ndc80 complexes per CENP-C, about a

	third of these complexes were stable, as evidenced by Ndc80-GFP brightness of the CENP-C/Mis12/KNL1. When complex formation was assayed by incubating CENP-C clusters with a mixture of 100 nM Mis12/KNL1 and 200 nM Ndc80, the binding of Ndc80 molecules increased two-fold, whereas Mis12 recruitment to CENP-C was not affected. This result may indicate that Mis12/KNL1 and Ndc80 first form a complex in solution and then their complex binds to CENP-C particles. Thus, Ndc80 binding recruitment is sensitive to specific binding pathway and is stronger when Mis12 complex is present in a soluble form. Interestingly, the proportion of stably bound Ndc80 complexes remained unchanged regardless of recruitment pathway, and the mechanisms limiting formation of stable Ndc80 assembly with CENP-C/Mis12/KNL1 clusters are unknown. The poster will describe our current efforts to test the role of Aurora B kinase in regulating both initial binding of Ndc80 to CENP-C/Mis12/KNL1 and formation of their stable assemblies.
27.	**Atomistic details of the dynein motor mechanism revealed by molecular dynamics simulations** **Juan F. Marín**, Yale E. Goldman, Jodi A. Hadden-Perilla Cytoplasmic dynein, which travels along microtubules (MTs), is responsible for long-range trafficking of cargoes toward the cell center. Each dynein molecule contains two motor domains that convert the energy of ATP hydrolysis into a cycle of unbinding from and rebinding to MTs; the alternate stepping of the two motor domains allows dynein to "walk" processively, while towing cargo. The mechanism of dynein motility remains the least understood of the cytoskeletal motors. Here, we employ all-atom molecular dynamics (MD) simulations to develop an atomistic description of dynein's mechanical mechanism, beginning with characterization of the motor domain as a function of nucleotide state for distinct stages of the hydrolysis cycle.
28.	**Investigating Convergent Molecular and Cellular Signatures of Microtubule Instability in Amyotrophic Lateral Sclerosis** **Kaya J.E. Matson** & Erika L.F. Holzbaur Amyotrophic lateral sclerosis (ALS) is a devastating disease that affects motor neurons in the brain and spinal cord, leading to progressive muscle weakness and paralysis. There are over 50 genes linked to ALS, and although they possess varied functions within cells, they all ultimately contribute to the degeneration of axons in motor neurons. A shared feature across ALS patients is the aggregation of proteins; aggregates of the RNA-binding protein TDP-43 are present in 97% of patients, while approximately 2% exhibit aggregates of FUS. TDP-43 dysfunction has been linked to microtubule instability and presynaptic loss at the neuromuscular junction through alternative splicing of the microtubule-binding protein, Stathmin-2 (STMN2). Given the importance of the microtubule cytoskeleton for axonal trafficking and function, microtubule instability may be a key contributor to axonal instability in ALS. Here, we will address whether distinct ALS-related mutations converge on a pathway promoting microtubule instability in axons due to inappropriate RNA regulation caused by TDP-43 or FUS inclusions, leading to axon degeneration in motor neurons. Using human iPSC-derived motor neurons with ALS-related mutations, I will first examine whether there are convergent molecular signatures via RNA sequencing. Next, I will examine cellular features using live imaging and function assays to determine whether these ALS-related mutations share the same vulnerability to cellular stressors. The intersection of these aims will help to identify convergent pathways of axonal instability and cellular pathology in ALS, potentially leading to the development of novel therapeutic interventions that target multiple genes and cellular processes simultaneously. Overall, this proposal addresses the multigenic nature of ALS, seeking to identify convergent molecular or cellular signatures related to microtubule dynamics that lead to neurodegeneration.

29. **Structural and Biochemical Effects of Missense MyBP-C Mutations on the Myofilament in Human Hypertrophic Cardiomyopathy**

McAllister, C.M., Jani, V., Nissen, D. Ma, W., Irving, T., Kass, D.A., Day, S.M.

While the disease mechanisms of truncating MyBP-C variants in HCM have been well defined, the disease mechanisms by which missense MyBP-C variants cause HCM are largely unknown. Recent studies have shown that unlike truncating variants which have a reduction in the level of MyBP-C, missense MyBP-C properly incorporates into the sarcomere at the correct protein abundance. The consequences of the presence of missense MyBP-C in the myofilament on myosin have not been explored and could have major implications for the treatment of HCM patients with missense variants in MyBP-C. First, we performed small angle X-ray diffraction (X-rD) on human HCM myectomy tissue collected from patients with missense MyBP-C. When nonfailing tissue was moved from resting (pCa 8) to activating (pCa 5) calcium conditions we observed an increase in the I1,1/I1,0 equatorial intensity ratio indicating that myosin has moved away from the thick filament backbone and adopted an active conformation close to the thin filament. However, in MyBP-C missense tissue I1,1/I1,0 is unchanged indicating that under activating conditions myosin remains in the inactive conformation suggesting the calcium response of the think filament is blunted. We next performed Mant-ATP assays to determine if the structural inactivation of myosin in missense tissue observed by X-rD is mirrored in the biochemical state. Previous studies have shown that in truncating HCM hearts there is a reduction in the amount of myosin in the slow energy-consuming biochemical state (SRX). However, we have observed that missense hearts do not undergo this reduction and have the same level of myosin in the slow ATP turnover state as nonfailing hearts. Treatment with the myosin modulator mavacamten increased the percentage of SRX myosin in both truncating and missense hearts. Last, we performed contractile assays to determine how missense variants affect heart muscle contraction. Our tension-calcium experiments have revealed that both truncating and missense hearts have a reduced maximum force generation (Tmax) compared to nonfailing hearts, with this reduction being greater in missense hearts. Missense hearts also exhibited an increase in calcium sensitivity. Treatment with Mavacamten caused a farther reduction in Tmax and rescued the increased calcium sensitivity observed in missense hearts.

30. **Investigating how map7 coordinates motor recruitment to regulate transport selectivity at branch junctions**

Elizabeth R. Moese, Stephen R. Tymanskyj, and Le Ma

Proper development and function of neurons requires tightly regulated microtubule (MT) -based transport of various cargo from cell bodies to synaptic terminals. Improper transport is implicated in various neurological diseases. Neurons have elaborately branched axons creating complex connections. Intracellular transport at axon branch junctions requires tight regulation of cargos, as suggested by recent discovery of selective transport in cultured (dorsal root ganglion) DRG neurons. Anterograde transport of lysosomes displayed preference for longer branches or branches with a dynamic growth cone. Furthermore, this selectivity is differentially regulated for various cargos and mediated by kinesin-3 motors. However, it is unknown what local mechanisms at branch junctions are mediating transport selectivity. Interestingly, MAP7 is a MT associated protein enriched at branch junctions in embryonic DRG neurons. *In vitro*, MAP7 recruits kinesin-1 to MTs but inhibits other kinesins (including kinesin-3) from MT binding. Transport of cargos such as lysosomes when MAP7 is overexpressed displayed increased run time but decreased velocity indicating kinesin-3 MT binding is blocked. Therefore, we hypothesize that MAP7 is coordinating motor recruitment and transport selectivity at branch junctions. To test this hypothesis, we examined MAP7 knockout neurons and preliminary data showed an increase in lysosome velocity consistent with the idea that kinesin-3 runs at a faster rate and locally MAP7 is coordinating motor recruitment. Moreover, we examined how MAP7 phosphorylation might affect transport selectivity at branch junctions as MAP7 contains phosphorylation sites in its P-domain. Mutating SP/TP sites in the P-domain, affects MT binding and axonal localization suggesting

	phosphorylated MAP7 may contribute to changes in transport at branch junctions, specifically by increasing kinesin-3 transport through branches. Finally, developing an expansion microscopy technique we will examine MAP7 localization at branch junctions at a higher resolution. By parsing out the relationship between the regulation of MAPs and intracellular transport we will begin to understand how regulated transport supports axon development and maintenance.
31.	**Effects of fibroadipogenic progenitor cells and intramuscular adipose tissue on skeletal muscle health and regeneration** **Natalie Moore**, Cindy Lu, Sarah Traynor, Carmen Flesher, David Merrick Skeletal muscle injury is a common ailment that affects millions of people worldwide. Muscle regeneration after injury is mediated by tissue-resident fibroadipogenic progenitor cells (FAPs). These fibroblast-like mesenchymal progenitor cells, while incapable of differentiating down a muscle lineage, support muscle regeneration and are capable of differentiation into adipocytes to form intramuscular adipose tissue (IMAT). IMAT often infiltrates muscle tissue in response to perturbations such as injury and is correlated with defects in muscle health and function, but a full understanding of the role that IMAT plays in either supporting or impeding muscle regeneration after injury and the mechanisms by which this occurs is lacking. Furthermore, understanding the role of FAPs and their transition from fibroblast to adipocyte will be key to elucidating their role in supporting myogenesis. Using single nucleus RNA sequencing, I found that FAPs adopt a unique transcriptional identity immediately following injury, including upregulation of a disintegrin and metalloprotease 12 (ADAM12). ADAM12 is an extracellular matrix protease capable of cleaving pro-growth factors including IGF1, which is known to support muscle growth. While FAPs have been demonstrated to enhance satellite cell myogenesis in a co-culture environment, I found that IGF1 treatment alone is capable of stimulating FAP differentiation into adipocytes *in vitro*. These data suggest a potential mechanism by which FAPs support muscle regeneration after injury via a local increase in the bioavailability of IGF1, which may also act in an autocrine fashion to promote FAP adipogenesis.
32.	**Maximum-likelihood Analysis of Single-Molecule Data on Elongation in an In Vitro Eukaryotic Translation System** Clark Fritsch, Arpan Bhattacharya, Martin Ng, Hong Li, **Philip C. Nelson**, Barry S. Cooperman, Yale E. Goldman Single-molecule fluorescence resonance energy transfer yields rich datasets that can be used to interrogate the mechanisms of the eukaryotic ribosome. Traditional analyses, however, often reduce such data to first moments, for example, mean waiting time as a function of tRNA concentration. We outline a principled, maximum-likelihood approach that objectively fits the entire distributions of waiting times for a proposed kinetic model. Our approach is computationally inexpensive and amenable to bootstrap estimation of errors.
33.	**Targeting the Activin Type II Receptor Improves Quality of Weight Loss Driven by GLP-1 Receptor Agonism** **Elizabeth Nunn**[1,2], Natasha Jaiswal[1,2], Matt Gavin[1,2], Karima Drareni[1,3], Ryan Calhoun[1,3], Joseph Baur[1,2], Patrick Seale[1,3], Paul M. Titchenell[1,2*] [1]Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [2]Department of Physiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; [2]Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Obesity is a chronic, multifactorial disease that continues to rise in prevalence worldwide, and common lifestyle interventions such as alterations to diet and levels of physical activity often fail to help individuals lose and, importantly, sustain significant amounts of weight loss. The recent FDA approval of glucagon-like polypeptide 1 (GLP-1) receptor agonist semaglutide represents a significant breakthrough in effective pharmacological interventions for weight loss. In clinical trials, high-dose weekly injections led to mean total body weight loss of 14.9% over 68 weeks in adults, however, roughly 40% of this weight loss is due to loss of lean mass. Proposed treatment strategies that seek to improve the quality of weight loss, or composition lean vs. fat mass lost, may help to improve long-term sustainability of weight loss. Targeting signaling pathways regulating the size of skeletal muscle is a promising approach to modulating body composition during weight loss; signaling through the activin type II receptor (ActRII) or through the PI3K/Akt/mTOR axis works to promote or oppose skeletal muscle atrophy, respectively. In this study, we utilized a combined treatment strategy of anti-ActRII antibody bimagrumab and GLP-1 receptor agonist semaglutide to evaluate changes in body composition and systemic physiology in a mouse model of obesity, as well as muscle-specific deletion of Akt to elucidate the signaling pathways required for skeletal muscle hypertrophy resulting from blockade of ActRII signaling. We show that bimagrumab induces a nearly 10% increase in lean mass weight in both lean and obese mice over a two-week period, and in obese mice, treatment with bimagrumab plus semaglutide successfully prevents loss of lean mass while leading to fat mass loss greater than with semaglutide alone. These results indicate that targeting ActRII improves body composition during weight loss driven by GLP-1 receptor agonism. In future studies, we will evaluate the effects of ActRII blockade on energy expenditure and its potential to more effectively maintain weight loss long-term and further explore the mechanism of skeletal muscle hypetrophy.

34.

Bni5 links myosin-II to septins at the cell division site to promote retrograde actin cable flow and the robustness of cytokinesis

Hiroki Okada[1,*], Xi Chen[1], Kangji Wang[1], Joseph Marquardt[1,2], and Erfei Bi[1,*]

[1]Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
[2]Current affiliation: Department of Biology, Western Kentucky University, Bowling Green, KY

Bni5 is known to be the sole linker between myosin-II and the septin hourglass at the division site before anaphase in budding yeast, but the mechanisms underlying its interactions and functions remain unclear. Guided by an AlphaFold-predicted structure, we conducted a comprehensive structure-function analysis on Bni5, and found that specific coiled-coil regions at the N- and C-terminal ends of Bni5 mediate its interactions with Myo1 (myosin-II heavy chain), Elm1 (septin-associated kinase), and septins whereas its middle-disordered region regulates its timely removal from the division site, which, in turn, controls the timely remodeling of the septin hourglass into a double ring. We also found that Bni5 tethers Myo1 to the septin hourglass to facilitate retrograde actin cable flow, which contributes to asymmetric segregation of protein aggregates during bud growth. This tethering mechanism also leads to an increase of Myo1 at the division site, which endows cytokinesis with the robustness against different insults. Our study establishes a biochemical pathway of septin-Bni5-myosin-II at the division site, which provides a model for mechanistic understanding of the positioning and contribution of myosin-II to retrograde actin flow in mammalian cells.

35.

Structure of INF2 bound to F-actin reveals insight into processive elongation by formins

Nicholas J. Palmer, Malgorzata Boczkowska, Grzegorz Rebowski, and Roberto Dominguez

The actin cytoskeleton is a tightly controlled and orchestrated process in cells which plays a role in many functions within the cell. Formins are a diverse class of actin binding proteins that control the nucleation

and elongation of actin filaments. Key to formin's function is their ability to remain processivity bound to the fast-growing barbed end. The mechanism behind formin processivity is unknown. Inverted formin 2 (INF2) is a formin which in addition to barbed end elongation has the unique functions of actin severing and depolymerization. Here we use cryo-electron microscopy to determine the structure of INF2 in its native states bound to actin filaments. We find multiple conformations of INF2's FH2 domains bound to the core of actin filaments, which give key insights into the mechanisms of barbed end elongation. In addition, we leverage INF2's severing mechanism to determine a low-resolution barbed end structure of an FH2 domain. Further biochemical and cell studies based on these structures will strengthen our understanding of formin regulation of the actin cytoskeleton.

36. **Myosin-1C competes with tropomyosin 3.1 for binding to actin filaments**

Luther W. Pollard, Malgorzata Boczkowska, Roberto Dominguez, E. Michael Ostap

The expression of approximately 40 different tropomyosin (Tpm) isoforms confers different identities upon actin filaments, resulting in subcellular compartments with different filament dynamics and morphologies. Strikingly, these compartments have been shown to have differential selectivity for members of the myosin superfamily. For example, several Tpm isoforms are known to inhibit class-I myosins while activating class-II myosin motility. The mechanism of Tpm-myosin selectivity is unknown. It is possible that the exclusion of myosin isoforms results from steric blocking due to structural differences at the Tpm-actin binding site. Alternatively, differential regulation could be due to the intrinsic kinetic properties of myosins, and their ability to switch on the dynamic tropomyosin-actin filament. Here, we investigate the mechanism of Myo1C inhibition by Tpm3.1, a short Tpm isoform abundantly expressed in non-muscle cells. We show that filament gliding in the presence of Tpm3.1 requires higher Myo1C concentrations attached to coverslips than necessary for undecorated actin gliding. Tpm3.1 also reduces the number of filaments bound by Myo1C as a function of increasing ionic strength in the motility assay. Actin gliding speeds are substantially inhibited in the presence of saturating Tpm3.1; however, motility does occur. In contrast, we found that Tpm3.1 completely inhibits the actin-activated steady-state ATPase activity of Myo1C. To determine if the inhibitory effect is due to steric or kinetic differences, we performed cosedimentation assays of Tpm3.1-bound actin and Myo1C in the absence of ATP and found that high Myo1C concentrations displace Tpm3.1 from the actin pellet, showing that Myo1C and Tpm3.1 actin-binding are mutually exclusive. Together, these data suggest that the mechanism of reduced gliding velocity is due to a reduction in the number of heads bound to filaments as a result of steric blocking by Tpm3.1. We propose that high local enrichment of Myo1C, such as on the coverslip surface in motility assays, is enough to outcompete some of the Tpm3.1, thus enabling sub-optimal motility. One possibility is that Myo1C enriched at membrane surfaces alongside the dynamic assembly of branched actin networks can exclude Tpm3.1 from these networks. This work was supported by NIH grant 5R37GM057247 and NSF grant CMMI 15-48571 to EMO.

37. **Spatially Regulated Genome Activation is Required for Embryonic Quality Control in Vertebrate Embryos**

Wenchao Qian, Hui Chen, and Matt Good

During early embryogenesis, cells undergo divisions without concurrent growth. Zygotic genome activation (ZGA) is triggered when cell sizes are sufficiently reduced, and ZGA is required for downstream development. In *Xenopus* embryos, ZGA initiation is linked to the reduction to a critical size threshold, with small animal pole cells activating their genomes before large vegetal pole cells. This gradient of cell sizes is conserved among amphibians and lampreys, but the significances of the gradient and the coupled pattern of genome activation remain unclear. To address this, we spatially inverted the pattern of genome activation in *Xenopus* embryos and evaluated its developmental consequences. Using a temperature controller, we applied a temperature gradient to *Xenopus* embryos during the blastula

	stage. By specifically cooling the top, we slowed down the cell division in the animal pole, resulting in an inverted cell size gradient and delayed genome activation in this region. Strikingly, embryos with significant delays in animal pole cell division underwent cell death at the early gastrula stage, resembling the embryonic quality control pathway where apoptosis is activated following early stress exposure. Active Caspase 3 staining revealed an accumulation of apoptotic cells in the blastocoel of affected embryos. To determine if delayed animal pole transcription, but not changing of cell sizes, triggered the apoptosis pathway, we selectively injected transcription inhibitors into animal pole cells. This leads to the release and spread of apoptotic cells, similar to embryos treated with the temperature gradient. However, administering caspase inhibitors eliminated the population of apoptotic cells and rescued the embryos from rapid death. These findings led us to hypothesize that an animal hemisphere-expressed molecule might inhibit apoptosis activity. Through literature reading, we identified PDGF (Platelet-Derived Growth Factor) protein as an animal cap- expressed molecule known to inhibit apoptosis in mesoderm cells and facilitate their migration. Intriguingly, injection of PDGF protein into the blastocoel reduced the apoptosis activity caused by animal-pole-only transcription inhibitions, allowing further embryonic development. Our findings emphasize the critical role of early genome activation in the animal pole in embryonic quality control. We propose that PDGF protein serves as a vital component to sense the proper pattern of zygotic genome activation and preventing cell death. This study advances our understanding of the intricate regulatory mechanisms governing early embryogenesis and sheds light on the significance of spatially controlled genome activation in ensuring proper embryonic development and viability.
38.	**Interaction between the mitochondrial adaptor MIRO and the motor adaptor TRAK** Elana Baltrusaitis*, **Erika Ravitch***, and Roberto Dominguez * these authors contributed equally MIRO (mitochondrial Rho GTPase) consists of two GTPase domains flanking two Ca^{2+}-binding EF-hand domains. A C-terminal transmembrane helix anchors MIRO to the outer mitochondrial membrane, where it functions as a general adaptor for the recruitment of cytoskeletal proteins that control mitochondria dynamics. One protein recruited by MIRO is TRAK (trafficking kinesin-binding protein), which in turn recruits the microtubule-based motors kinesin-1 and dynein-dynactin. The mechanism by which MIRO recruits TRAK to mitochondria is not well understood. Here, we map and quantitatively characterize the interaction of human MIRO1 and TRAK1 and test its potential regulation by Ca^{2+} and/or GTP binding to MIRO1. TRAK1 binds MIRO1 with low micromolar affinity. The interaction was mapped to a fragment comprising MIRO1's EF-hands and C-terminal GTPase domain and to a conserved stretch of amino acids within TRAK1 residues 394-434, immediately C-terminal to the Spindly motif. MIRO1's EF-hands bind Ca^{2+} with dissociation constants (K_D) of 3.3 μM and 130 nM. This suggests that under normal cellular conditions, where the Ca^{2+} concentration ranges from 0.1-0.2 μM, one EF-hand may be constitutively bound to Ca^{2+}, whereas the other EF-hand binds Ca^{2+} in a regulated manner, depending on its local concentration. Yet, the MIRO1-TRAK1 interaction is independent of Ca^{2+} or GTP binding to MIRO1. The interaction is also independent of TRAK1 dimerization, such that a TRAK1 dimer can be expected to bind two MIRO1 molecules on the mitochondrial surface.
39.	**Computational model of myosin-I interactions with branched actin networks** **David M. Rutkowski** , Mengqi Xu, E. Michael Ostap, Dimitrios Vavylonis Myosin I's are found near sites of branched actin nucleation and growth, but how myosin-I motor activity works together with actin network growth is still largely unclear. We investigated branched actin network growth in the presence of myosin-I with computational modeling. The model was developed in parallel to an *in-vitro* actin-based motility system where branched actin network was

nucleated around a bead surface coated with the WCA domain of N-WASP and myosin 1D, in the presence of Arp2/3 complex and capping protein (CP). In our model, semiflexible actin filaments are represented as particles connected by springs that polymerize at their barbed ends and push against a spherical bead according to Brownian-ratchet-type force-elongation relationship. Myosin is assumed to exert a tangential force on actin filaments in close proximity to the bead. Spontaneous filament nucleation and branching at 70° angles occurs close to the bead; elongation stops by capping when filaments reach a specified length. Excluded volume interactions prevent filament crossing. By allowing filaments to break or debranch above a certain tensile force or branch angle threshold, we find that the branched networks growing around the bead can crack open, leading to symmetry breaking and bead propulsion. In agreement with experiment, our simulations at a reference branch filament length show that myosin forces lead to a less dense actin network while leaving the bead velocity unaffected. Furthermore, while actin polymerization and squeezing propels the bead in simulations without myosin, the myosin force dominates the propulsive force when sufficiently high tangential myosin forces are applied. Consistent with experiments, we find that for shorter filaments (higher CP) myosin hinders symmetry breaking while for longer filaments (lower CP) myosin aids it. Strikingly, and in agreement with experiment, we find that symmetry breaking can be achieved with myosin alone after halting actin polymerization. Overall, our simulations and experiments suggest myosin is a main contributor to branched actin network growth, affecting both the network morphology and the network's ability to produce force.

40. **Pan-tissue scaling of stiffness versus fibrillar collagen reflects cellular contractile-strain driven collagen degradation**

K. Saini[1], S. Cho[1], M. Tewari[1], A. Jalil[1], M. Wang[1], S. Belt[1], A. Kasznel[1], K. Yamamoto[2], D. Chenoweth[1] and D. E. Discher[1,*]

[1] University of Pennsylvania, Philadelphia, PA 19104, USA; [2] University of Liverpool, UK

Polymer network properties such as stiffness often exhibit characteristic power laws in polymer density and other parameters. However, it remains unclear how cell and molecular mechanisms contribute towards homeostatic differences among animal tissues and whether diverse tissues, composed of many distinct polymers, exhibit such scaling. Here, we examined many diverse tissues from adult mouse and embryonic chick to determine if stiffness (E_{tissue}) follows a power law in relation to the most abundant animal protein, Collagen-I, even with diverse molecular perturbations. We quantified fibrillar collagen in intact tissue by label-free second harmonic generation (SHG) imaging and from tissue extracts by mass spectrometry (MS), and collagenase-mediated decreases were also tracked. Pan-tissue power laws for tissue stiffness versus Collagen-I levels measured by SHG or MS exhibit sub-linear scaling that aligns with results from cellularized gels of Collagen-I but not acellular gels. Interestingly, inhibition of cellular contractile strains fits the scaling, and combination with inhibitors of matrix metalloproteinases (MMPs) show collagenase activity is strain - not stress- suppressed in tissues, consistent with past studies of gels and fibrils. Beating embryonic hearts and tendons, which differ in both collagen levels and stiffness by >1000-fold, similarly suppressed collagenases at physiological strains of ≈5%, with fiber-orientation regulating degradation via strain-dependent collagen molecular conformation. Scaling of E_{tissue} based on 'use-it-or-lose-it' kinetics provides insight into scaling of organ size, microgravity effects, and regeneration processes while suggesting contractility-driven therapeutics.

41. **Comprehensive analysis of blood recalcification using the fluorescent calcium indicator Rhod-5N**

Taisia O. Shepeliuk[1], Anastasia A. Masaltseva[1,2], Fazoil A. Ataullakhanov[1,2], Ekaterina L. Grishchuk[1]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, Moscow, Russia.

Clinical coagulation testing and research studies often commence by chelating Ca^{2+} ions through the addition of anticoagulants such as sodium citrate. Subsequently, these ions are reintroduced during blood recalcification. The precise restoration of the physiological concentration of Ca^{2+} ions, up to 1.1-1.3 mM, is crucial for normal coagulation. However, there exists a significant disparity in the available protocols for controlling calcium concentration in blood, which hinders the comparison of blood coagulation results across different studies. To address this limitation, we conducted a systematic investigation of blood recalcification using mathematical modeling and direct measurements in blood and plasma. We show that free calcium concentration in both blood and plasma can be accurately determined using a fluorescence calcium indicator, Rhod-5N, owing to its low apparent dissociation constant (1.06 mM). Utilizing a theoretical model of citrated blood/plasma recalcification, we calculated the relationship between the concentration of free Ca^{2+} ions in blood/plasma and the concentration of externally added calcium chloride solution. These predictions were then confirmed using Rhod-5N sensors and traditional calcium electrode measurements. Our study highlights the importance of considering hematocrit levels, which can vary up to 30% in healthy individuals and even more dramatically in patients. Hematocrit level in the donor's blood should be taken into account when recalcification is carried out in plasma. However, these variations are naturally accounted for when recalcification is performed in whole blood. Finally, we demonstrate that Rhod-5N can be employed to monitor blood recalcification in microfluidic chambers. Using this approach, we estimated the effective diffusion coefficient of Ca2+ ions in blood to be 8.6×10^{-10} m2/s. Collectively, this newfound knowledge should enhance the precision of blood recalcification in batch processes and facilitate the rational design of microfluidic devices equipped with on-chip recalcification capabilities.

42. **Stress granules as a mechanism of translational control in cardiomyocytes**

Katey R. Stone, Emily A. Scarborough, Benjamin L. Prosser

The heart is largely made up of post-mitotic, non-regenerative cardiomyocytes (CM) that require tight coupling of translational control and proper proteostasis to constantly respond to translational needs over an organism's lifetime. However, the mechanisms by which CMs spatially and temporally control translation are largely unknown. Stress granules (SG) are non-membranous phase-separated granules containing mRNA and mRNA binding proteins. SGs have been shown to function in translational downregulation in other cell types. Although some studies suggest that SGs may assemble in neonatal CMs, the occurrence and function of SGs in mature myocardium have not been well-studied. We hypothesize that CMs use the assembly and disassembly of SGs to tune translational activity for general proteostasis and upon stress to accommodate hypertrophy. To test this, we use immunofluorescence (IF) and RNA fluorescence in situ hybridization (FISH) to visualize mRNA and RNA binding proteins (RBP) in neonatal and adult CMs. Additionally, methionine depletion and methionine analogs in combination with click-chemistry are used to measure translation. We use overexpression of SG-associated RBP G3BP1 and heat shock as methods of inducing stress and causing SG assembly. We find that neonatal CMs form distinct puncta that contain G3BP1 and polyA mRNA in some cells under control conditions and in all cells after heat shock. We find that neonatal CMs overexpressing GFP-G3BP1 at a high level form distinct puncta that colocalize with SG-associated RBP PABPC-1 and show a drastic reduction in translation, while those expressing at a low level do not form puncta and show no change in translation compared to those not expressing GFP-G3BP1. We find that mature adult CMs form distinct puncta in G3BP1 and polyA mRNA as well as show reduced translation following heat shock. Together, these data indicate that CMs form SGs that incorporate canonical SG proteins, and when SGs are present, rates of translation are decreased. In the future, we will further identify the composition of SGs in CMs and the stressors that induce their assembly, and determine their role in modulation of translation and subsequent cardiac hypertrophy.

43. **Microtubule to nucleoskeleton force transmission, but not dynamic strain transfer, drives nuclear damage in LMNA cardiomyopathy**

Daria Amiad-Pavlov[1], **Carmen Suay-Corredera[1]**, Julie Heffler[2], Keita Uchida[1], Jan Lammerding[2], Benjamin Prosser[1]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania
[2]Weill Institute for Cell & Molecular Biology, Cornell University

The adult cardiomyocyte has evolved to continuously generate and withstand substantial shortening and stretch cycles. This demanding and adaptive mechanical environment is supported by a complex cytoskeletal network that links the sarcomere to the nucleus. The dynamic and basal forces from the surrounding microtubule, intermediate filaments and sarcomere networks are transmitted into the lamina and chromatin at the inner nuclear membrane through the linker of the nucleoskeleton and cytoskeleton (LINC) complex. Intriguingly, while human mutations in the cytoskeletal, LINC, and lamin proteins lead to cardiomyopathies, disruption of the LINC complex is cardioprotective in myopathies driven by mutations in lamin A. The contractile forces in the adult muscle have been hypothesized to induce damage to nuclei with weakened lamina, and decoupling force transmission to the nuclei confers mechanical protection, but the mechanism is not known. Here, we investigated the cytoskeletal to nuclear strain transfer in the adult beating cardiomyocyte and specifically perturbed microtubules, LINC complex proteins and the lamina. We demonstrate reduced dynamic strain transfer into the nucleus with in vitro disruption of the LINC complex, and increased dynamic nuclear strain transfer in LMNA N195K mouse cardiomyocyte. However, cardiac specific in vivo LINC disruption in the LMNA N195K mouse model did not restore the dynamic nuclear strain transfer, despite increased survivability. Further analysis of the LMNA N195K mouse model suggests that the effects of in vivo LINC disruption in the cardiomyocytes are dominated by decoupling of the nucleus from the dense perinuclear microtubule cage, supporting the hypothesis that basal microtubule forces on the weakened lamina are the main driver of nuclear damage in cardiomyocytes of this laminopathy model. These findings provide better understanding of the microtubule driven nuclear damage in the LMNA N195K laminopathy, and the mechanism of cardioprotective, LINC mediated decoupling of the nucleus from the microtubules. Elucidating the unique mechanical interplay between the cytoskeleton and the nuclear lamina in the mature heart, and how it is shifted in physiological and pathological adaptation can help design specific therapies for laminopathy and envelopathy driven cardiomyopathies.

44. **Clustering of the scaffolding protein CENP-T activates recruitment of Ndc80 complexes to assemble a functionally active outer kinetochore**

Ekaterina V. Tarasovetc[1], Gunter B. Sissoko[2], Aleksandr Maiorov[1], Iain M. Cheeseman[2], Ekaterina L. Grishchuk[1]

1. Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2. MIT Department of Biology, Cambridge, MA; Whitehead Institute for Biomedical Research, Cambridge MA

Proper kinetochore assembly requires binding of multiple copies of different proteins in an ordered and localized manner. In particular, the human kinetochore scaffolding protein CENP-T recruits two Ndc80 molecules, which are required for stable interaction with spindle microtubules. However, when the CENP-T-kinetochore localization domain is removed, individual CENP-T molecules do not associate robustly with Ndc80 when present in the cytoplasm, highlighting the importance of the centromere localization of CENP-T. We hypothesized that clustering of CENP-T molecules at centromeres plays a role in Ndc80 recruitment. To test this, we created genetically-encoded 60-subunit clusters of CENP-T's outer kinetochore recruitment domain and expressed them in human cells. Clustered CENP-T, but not monomeric CENP-T^{1-242}, robustly recruited Ndc80 and other outer kinetochore proteins. Moreover,

	complexes formed by CENP-T clusters and outer kinetochore components isolated from cells bind to microtubules and move processively with dynamic microtubule tips. To investigate the mechanism that leads to assembly of a functionally active outer kinetochore on clustered CENP-T, we tested whether this phenomenon can be recapitulated using recombinant components. We used a real time TIRF fluorescence assay to monitor interactions between GFP-tagged Ndc80 and CENP-T$^{1\text{-}242}$ present in either clustered or monomeric forms. Both forms of CENP-T bind to two Ndc80 complexes, but we found that Ndc80 molecules dissociate significantly faster from monomeric compared to clustered CENP-T. In addition, the stability of CENP-T-Ndc80 binding increases over time, indicating a "maturation" process of Ndc80 binding sites on CENP-T. Such maturation was concurrent with the presence of multiple weakly-bound Ndc80 molecules around CENP-T clusters. The formation of such molecular clouds was not observed with monomeric CENP-T molecules, which experienced much slower maturation compared to clustered CENP-T. Thus, the enhancement of Ndc80 recruitment and stabilization of its binding to CENP-T is an intrinsic feature of CENP-T clusters and is associated with different kinetics of Ndc80 binding and maturation. We propose that, in cells, these molecular mechanisms promote efficient and stable binding of Ndc80 and other outer kinetochore components specifically at the centromere loci, while avoiding interactions between the soluble components in the cytoplasm.
45.	**Possible Regulation of Myosin-19 by the E3-Ligase MARCH5** **Cameron P. Thompson**, Erika L.F. Holzbaur, E. Michael Ostap Myosin-19 (Myo19) is a mitochondrially-localized, actin-based motor that has been shown to play a role in regulating mitochondrial morphology and health. Previous work has shown that Myo19 localization to the mitochondria is tightly controlled, and motors not bound to the organelle are largely degraded. The mechanisms for control of this system have not been fully discerned. Initial experiments have shown that Myo19 is ubiquitylated in the cell, in agreement with previously reported whole proteome ubiquitylation analysis. Previous work from our lab found that the E3-ligase MARCH5 co-purifies with Myo19, inspiring our further investigation into the potential role of MARCH5 in Myo19 regulation. Through knockdown experiments, we found that both total cellular and mitochondrially localized protein levels of endogenous Myo19 are not changed by loss of MARCH5. Interestingly, MARCH5 expression levels affect the presence of a proteolyzed subpopulation of Myo19 that is ~20kDa smaller than the full-length motor. Furthermore, it appears that the proteolysis is occurring at the N-terminus of the motor which would render it unable to bind actin filaments. We hypothesize that MARCH5 is involved in the partial proteolytic regulation of Myo19 that produces a polypeptide incapable of actin binding. We speculate that this regulatory pathway could explain how the cell tightly controls the levels of functional Myo19 on the mitochondria, therefore ensuring proper organelle morphology and health.
46.	**A novel cytoskeleton-based pathway regulates mitochondrial dynamics and energetics in skeletal muscle** **Kayleigh Voos**, Joyce Tzeng, Trevor Pharr, Priya Patel, Sophie Rubinsky, Grace Choi, and Damaris Lorenzo Regulation of mitochondrial dynamics is key for bioenergetic homeostasis. Dysregulation of mitochondrial fission and fusion in skeletal muscle (SKM) promotes metabolic deficits. The cytoskeleton is a key modulator of mitochondrial dynamics. However, the cytoskeletal mechanisms that regulate mitochondrial dynamics are elusive. Interestingly, human variants in the cytoskeleton-associated scaffolding protein Ankryin-B (AnkB), are associated with cardio-metabolic disease, and cause age-dependent metabolic syndrome in mice. However, the metabolic roles of AnkB in SKM have not been characterized. Using a conditional knock-out mouse model that selectively lacks AnkB in SKM (SKM-AnkB-KO mice), we identified a novel role of AnkB in regulating SKM energetics through association with mitochondria. We found that muscle from these mice have disorganized microtubule

	arrangement. Additionally, SKM-AnkB-KO mice exhibit reduced exercise capacity while their muscle have enlarged mitochondria, suggesting alterations in mitochondrial dynamics. Interestingly, protein levels of dynamin-like protein 1 (DRP1), a major mitochondrial fission mediator, are reduced in different muscle types lacking AnkB. Moreover, we found that AnkB interacts with DRP1. Together, these data suggest AnkB, through interaction with DRP1 and organization of the microtubule cytoskeleton, plays a direct role in mediating mitochondrial fission in SKM, and this directly impacts whole muscle bioenergetic capacity.
47.	**Unravelling the mechanisms and evolution of a two-domain module in IQGAP proteins for controlling eukaryotic cytokinesis** **Kangji Wang**,[1] Hiroki Okada,[1] Carsten Wloka,[1,2] and Erfei Bi[1] [1]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania [2]Experimental Ophthalmology, Department of Ophthalmology, Charité - Universitätsmedizin Berlin, A Corporate Member of Freie Universität, Humboldt-University, The Berlin Institute of Health, Berlin, Germany The IQGAP family of proteins plays a crucial role in cytokinesis across diverse organisms, although the underlying mechanisms are not fully understood. In this study, we demonstrate that IQGAPs in budding yeast, fission yeast, and human cells use a two-domain module to regulate their localization as well as the assembly and disassembly of the actomyosin ring during cytokinesis. Strikingly, the Calponin Homology Domains (CHDs) in these IQGAPs bind to distinct F-actin structures in the cell with varying specificity, whereas the newly identified domains downstream of the CHDs in these IQGAPs all target to the division site, but differ in timing, localization strength, and binding partners. We also demonstrate that human IQGAP3 acts in parallel to septins and myosin-IIs to mediate the role of anillin in cytokinesis. Collectively, our findings highlight the two-domain mechanism by which IQGAPs regulate cytokinesis in distantly related organisms as well as their evolutionary conservation and divergence.
48.	**Myosin-I facilitates symmetry breaking and promotes the growth of actin comet tails** **Menggi Xu**, Luther W. Pollard*, Grzegorz Rebowski, Malgorzata Boczkowska, Roberto Dominguez, E. Michael Ostap* Actin assembly stimulated by the Arp2/3 complex provides pushing forces for a variety of cellular processes. Type-I myosins (myosin-Is) are a class of actin binding motor proteins that are commonly found alongside Arp2/3-mediated branched actin networks at membrane interfaces and participate in actin-mediated force generation. Yet, the molecular effects of how myosin-Is interact with the actin network remain elusive. To further investigate the roles of myosin-I in actin-mediated force generation, we reconstituted an *in vitro* actin-based motility system, where branched actin networks were nucleated by Arp2/3 complex from a micron-sized bead surface coated with Arp2/3 activating factors. Actin filaments first formed a symmetric shell around the bead, which transitioned into a polarized comet tail after symmetry breaking and propelled the bead forward. We site-specifically coupled a range of densities of myosin-Is to the bead surface and assessed their effects on actin polymerization, network architecture, and symmetry breaking. We found that myosin reduced the density of actin network while maintaining comparable or even faster comet growth rates. Under conditions where actin shells were too dense to be fractured by polymerization forces alone, myosin promoted symmetry-breaking. Furthermore, when actin assembly was arrested around the bead surface before symmetry breaking, we found that myosin motor activity alone can break the sparse actin shell. By employing rigor myosin, we confirmed that these emergent effects primarily stem from the force-generation power-stroke of myosin-I, rather than its dynamic actin tethering properties. Our results suggest that myosin-I is likely involved in the branched actin assembly by facilitating more efficient force-production through its power-stroke.

49.

Single molecule FRET, anisotropy decay, and diffusion coefficient measured by multi-parameter confocal fluorescence spectroscopy

Him Shweta[2], **Amber Yanas**[1], Michael C. Owens[1], Yale E. Goldman[2] and Kathy Fange Liu[1]

1. Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
2. Department of Physiology, Pennsylvania Muscle Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Sex differences are pervasive in human health and disease. The most striking differences lie in the sex chromosomes, which encode a group of sex-specific protein homologs. Although the functions of the X chromosome proteins are well appreciated, how they compare to their Y chromosome homologs remains elusive. DDX3X and DDX3Y are one such pair of sexually dimorphic non-processive ATP-dependent RNA helicases. We previously demonstrates that DDX3Y forms larger RNA-dependent, phase-separated liquid condensates compared to DDX3X, which is possibly due to differences in kinetics and dynamic interactions with RNA and the fact that DDX3X has higher ATPase activity than DDX3Y. Here, we used multi-parameter confocal fluorescence spectroscopy to study helicase dynamics. We acquired ns time-resolved fluorescence anisotropy, diffusion coefficients, and FRET of Cy3- and Alexa647-labeled double-stranded RNA (dsRNA) upon addition of the helicases in the presence and absence of ATP. Freely diffusing Cy3- and Alexa647-labeled RNA duplexes show FRET efficiency (*E*) ≅ 0.25. With DDX3 protein bound, *E* ≅ 0.9, and when ATP is added to the RNA and DDX3 mixture, *E* ≅ 0.1. The time-resolved fluorescence anisotropy and population analysis of the doubled-labeled, donor-only and acceptor-only particles selected from the 2D histogram of *E* vs. stoichiometry (*S*) shows that this shift in ATP-dependent FRET change is associated with DDX3 binding to the RNA and partially unwinding the duplex. Fluorescence correlation spectroscopy (FCS) data shows markedly slowed diffusion rate (*D*) for RNA-DDX3X (~12 $\mu m^2/s$) and RNA-DDX3Y (~8 $\mu m^2/s$) compared to the dsRNA (~98 $\mu m^2/s$), suggesting that these RNA-helicase complexes form nano- sized clusters by accumulating multiple DDX3X or DDX3Y molecules at concentrations well below those required for forming frank condensates. A 10-fold change in *D* corresponds to an apparent 1,000-fold increase in volume! We find that these clusters promote efficient RNA unwinding activity. Collectively, the kinetics and smFRET data support that DDX3Y has weaker ATPase activity, leading to the less dynamic RNA-DDX3Y complexes. Decreased dynamics, in turn, may contribute to the weaker disassembly of DDX3Y condensates upon addition of ATP compared to DDX3X condensates.

50.

Residue-specific coarse-grained model of actin filaments

Shuting Zhang, Dimitrios Vavylonis

A challenge in understanding the molecular regulation of actin cytoskeleton is the development of computationally efficient and accurate models. We developed a coarse grained molecular dynamics model of actin filaments that incorporates residue-specific interactions through the C-alpha implicit-solvent model of Kim and Hummer (KH), modified to account for experimentally-determined actin-actin interactions. While the KH model correctly predicts the broad features of actin-actin interactions, it does not consistently maintain all long- and short-pitch contacts required for a right handed helix of actin subunits. We show the latter can be achieved by specifying additional specific interactions between subdomains 3 and 4, two pairs between the D-loop and the cleft of subdomains 1 and 3, and one pair for short-pitch interaction. We also give the actin subunits flexibility to tilt/open to mimic the transition between G- and F- actin forms. The modified KH model is able to capture the critical contacts in actin filament formation and stabilization and should be useful to understand interactions of actin filaments to side binding proteins.

NOTES

Sponsored by the
Physiological Society of Philadelphia
and the *Pennsylvania Muscle Institute*

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