SUMMARY OF POSTER ABSTRACTS

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Last Name	First Name	Institution	Mentor	ECI Session	Poster Session	Poster #	Abstract Category	Title
Aboelkassem	Yasser	University of Michigan - Flint			1	134	Regulation and Modulation of Contraction	Deep Learning as a Surrogate Myofilament Model: Predictive AI for Cardiac Sarcomere Dynamics
Ahlin	Casey	Rensselaer Polytechnic Institute	Douglas Swank		2	110	Other Topics in Myofilament Biology	Drosophila Aging Mirrors Human Sarcopenia: Age-Related Decline in Muscle Power and Mobility
Alekhina	Olga	University of Arizona			2	112	Other Topics in Myofilament Biology	Viral Vector Core Facility: accelerate your research with our reliable viruses
Altenburg	Ilse	Amsterdam University Medical Center	Diederik Kuster		1	91	Regulation and Modulation of Contraction	Developing single cell perfusion and contractility setup for cardiomyocytes to understand cell-to-cell variability in drug response
Amrine	Mackenzie	University of Colorado	Kathleen (KC) Woulfe		1	1	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	The interfibrillar mitochondrial metabolome in aged female hearts leads to a unique microenvironment and alters acylation of sarcomeric proteins
Ananthamohan	Kalyani	University of Arizona	Sakthivel Sadayappan		1	3	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Rbm24 Mediated Alternate Splicing of *MYBPC3* in Hypertrophic Cardiomyopathy
Baby	Akhil	University of Arizona	Sakthivel Sadayappan		2	74	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Slow MyBP-C in Skeletal Myogenesis: Exploring Novel Functions Beyond the Sarcomere
Barnett	Cara	Loyola University Chicago	Jordan Beach & Patrick Oakes		2	100	Myofilament-based Mechano-sensing	Mechanosensitive Myopathy: FHL1 Missense Variants Impair Strain Sensing Response
Barry	Meaghan	Johns Hopkins University	Anthony Cammarato & David Kass		1	7	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Phosphorylation of TnI-T181: Redefining HFpEF, model organisms, and potential therapeutic targets
Bodt	Skylar	Johns Hopkins University	Anthony Cammarato		1	9	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	The Impact of Myosin Modulators on Myofilament Dysfunction in Obese Heart Failure with Preserved Ejection Fraction
Bogaards	Sylvia	Amsterdam University Medical Center			2	114	Other Topics in Myofilament Biology	The replacement kinetics of the giant muscle protein nebulin are slow
Brudz	Vanessa	University of Guelph	John Dawson		1	11	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	ACTC1 A319_L320*del* : A Model Variant to Characterize Subdomain 3 Thin Filament Dysregulation in Cardiomyopathy Development
Brunello	Elisabetta	King's College London			1	93	Regulation and Modulation of Contraction	Molecular basis of length-dependent activation in cardiac muscle
Callahan	Damien	University of Oregon			2	76	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Post-Activation Potentiation and the Influence of Estrogen, Age and Regulatory Light Chain
Camillo	Jared Rafael	San Diego State University	Sanford Bernstein		1	13	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Biophysical, Biochemical, and Structural Analyses of Drosophila Cardiac Myosins and Effects of Dilated Cardiomyopathy Mutations
Campbell	Stuart	Yale University			1	15	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Consequences of a *TTN* Truncating Mutation on Active and Passive Mechanics of Human Engineered Heart Tissue
Chastain	Ronnie	Florida State University	Jose Pinto		1	17	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Investigation of DCM-mutation (*ACTC1* -E361G) with Blunted Response to β-Adrenergic Stimulation in Mice

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Chen	Yu-Chieh	Temple University	J. Travis Hinson		1	19	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	AAV-Mediated Troponin Replacement Reclassifies *TNNT2* Variants and Uncovers Myocardial Plasticity of Dilated Cardiomyopathy
Chesler	Naomi	University of California Irvine			1	95	Regulation and Modulation of Contraction	Modeling the Impact of Preterm Birth on Myocyte Crossbridge Kinetics and Force
Childers	Matthew	University of Washington	Michael Regnier	X	2	2	Myofilament Structure and Function	Modeling myosin's departure from the IHM with molecular simulation
Chinthalapudi	Krishna	The Ohio State University			2	4	Myofilament Structure and Function	Structural Polymorphism of the Cardiac Actin Y166C as a Molecular Basis for Hypertrophic Cardiomyopathy
Chitre	Kripa	Johns Hopkins University	Anthony Cammarato		2	6	Myofilament Structure and Function	Genetically edited insect flight muscle: a novel resource for recombinant sarcomeric actin production and *in vivo* investigation
Chung	Charles	Wayne State University			1	97	Regulation and Modulation of Contraction	Does Modifying Calcium Sensitivity Modify Mechanical Control (Strain Rate Sensitivity) of Relaxation?
Cizauskas	Hannah	Loyola University Chicago	David Barefield	X	1	5	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Aging exacerbates contractile dysfunction in a mouse model of atrial myopathy
Colson	Brett	University of Arizona			1	99	Regulation and Modulation of Contraction	Myosin-Binding Protein C N-Terminal Domains Slow Ca2+ Dissociation from Cardiac Thin Filaments
Craig	Roger	UMass Chan Medical School			2	8	Myofilament Structure and Function	Comparing Cryo-EM Structures of the Vertebrate Cardiac Muscle Thick Filament
Crawford	Rhiannon	Florida State University	Christopher Solis		2	10	Myofilament Structure and Function	In Silico Analysis of Conformational Dynamics of the Alpha-Actinin-2 Actin-Binding Domain in Response to Hypertrophic Cardiomyopathy Mutations
Crosby	Garrett	University of Arizona	Sakthivel Sadayappan		2	12	Myofilament Structure and Function	Pathophysiological Significance of Linker and Loop Motifs of Cardiac Myosin Binding Protein-C
de Tombe	Pieter	University of Florence			1	21	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Mutation-related efficacy of novel therapeutic approaches in hypertrophic cardiomyopathy
Delligatti	Christine	Johns Hopkins University	Anthony Cammarato		2	116	Other Topics in Myofilament Biology	Flying into the Future of Sarcopenia: A Proteomic Atlas of Aging *Drosophila melanogaster* IFMs Supports Time-Dependent Molecular and Structural Dysregulation of Skeletal Muscle
Demeulenaere	Sasha	Loyola University Chicago	Jordan Beach		2	132	Smooth Muscle and Non-Muscle Myosins	Disease-associated MYH11 tail variants disrupt smooth muscle myosin II filament assembly and contractility in vascular smooth muscle cells
Door	Michaela	University of Chicago	Jonathan Kirk		1	101	Regulation and Modulation of Contraction	Protein Tyrosine Phosphatase 1B as a Novel Regulator of Myofilament Function in Disease
DuVall	Mike	Edgewise Therapeutics			2	14	Myofilament Structure and Function	Chamber-Specific Responses to Length Dependent Activation in Porcine Myocardium
Dvornikov	Alexey	University of Arizona			1	103	Regulation and Modulation of Contraction	Effects of dextran in skeletal muscle with and without Myosin Binding Protein-C

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Engels	Nichlas	University of Arizona	Samantha Harris		1	105	Regulation and Modulation of Contraction	Loss of Myosin binding protein-C (MyBP-C) increases strain induced cross-bridge detachment in striated muscle
Farman	Gerrie	University of Arizona			2	16	Myofilament Structure and Function	Impact of Lmod2 on Thin Filament Structure
Fenwick	Axel	Johns Hopkins University			1	23	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Impaired Myofibril Relaxation in a Porcine Model of cTnI-Associated Hypertrophic Cardiomyopathy is Rescued with AAV Gene Therapy
French	Nathan	University of Michigan	Alison Vander Roest		1	107	Regulation and Modulation of Contraction	Effects of Myosin-Specific Pharmacologics on Cardiomyocyte Mechanical Behavior
Friedman	Clayton	University of Washington	Kai-Chun Yang & Chuck Murry		2	18	Myofilament Structure and Function	Rules of the Rod: *MYH7* Variant Effect Across the β-MHC Coiled-Coil Domain
Galli	Ricardo	National Institutes of Health	Carsten Bonnemann & Coen Ottenheijm	X	2	78	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Spontaneous oscillatory muscle contractions are from sarcomeric origin in novel *MYBPC1* variant associated with myogenic tremor
Garg	Ankit	Johns Hopkins Medicine		X	1	25	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Variants in skeletal muscle actin as potent disruptors of cardiac contractility
Gilbertsen	Lauren	University of Idaho	Daniel Fitzsimons		1	109	Regulation and Modulation of Contraction	Altered contractile dynamics underlie adaptations in cardiac function in the maturing porcine heart
Gohlke	Jochen	University of Arizona	Henk Granzier		2	20	Myofilament Structure and Function	Evolutionary and structural analysis reveals presence of novel domain elements in titin
Goluguri	Rama Reddy	Stanford University	James Spudich & Kathleen Ruppel		1	111	Regulation and Modulation of Contraction	Resolving ATP Turnover Kinetics and IHM Formation of β-Cardiac Myosin at the Single-Molecule Level
Gong	Henry	University of Chicago	Jonathan Kirk		2	22	Myofilament Structure and Function	Methylglyoxal induces sarcomere dysfunction and impairs relaxation in the heart
Greenman	Angela	University of Wisconsin-Madison	J. Carter Ralphe		1	27	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Altering the ratio of reduced to oxidized glutathione affects contractile kinetics in cardiomyocytes
Hale	Joshua	University of Arizona	Carl Tong		1	115	Regulation and Modulation of Contraction	Calcium-Independent Relaxation Enhancement is Impaired in Hearts with Diastolic Dysfunction
Han	Julia	University of Michigan	Alison Vander Roest		1	29	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Mant-ATP-Informed Patient-Specific Cardiac Mechanics in Hypertrophic Cardiomyopathy
Han	Seong-won	University of Arizona	Henk Granzier		2	80	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Characterizing Skeletal Muscle Dysfunction in Hereditary Myopathy with Early Respiratory Failure (HMERF): Insights from Human Muscle Biopsies
Hanft	Laurin	University of Missouri			1	117	Regulation and Modulation of Contraction	PKA phosphorylation of cTnI increases power output in non-phosphorylatable cMyBP-C permeabilized cardiac myocytes
Harriot	Anicca	Johns Hopkins University	Deok-Ho Kim		2	82	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Frailty-on-a-Chip: Developing a Microphysiological Model of Age-Related Skeletal Muscle Dysfunction

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Hauck	Garrett	University of Arizona	Jil Tardiff		1	31	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Small Molecule Compounds that Restore Cardiac Thin Filament Calcium Exchange Improve Myocellular Function in a Murine Model of Hypertrophic Cardiomyopathy
Heiser	Torri	University of Calgary	Walter Herzog	X	2	24	Myofilament Structure and Function	Energy Consumption After Active Stretch in Single Skinned Muscle Fibres: Titin or No Titin?
Hessel	Anthony	University of Muenster			1	119	Regulation and Modulation of Contraction	Increasing I-Band Titin Compliance Shifts the Structure and Function of Intact Rat EDL Muscle During Peak Twitch and Tetanic Contraction Towards a Less Active State
Hodgeman	Brook	University of Vermont	Wen Ma	X	2	118	Other Topics in Myofilament Biology	Data-Driven Mechanistic Modeling of Myosin Turnover and the Effects of Mavacamten
Hoffer	Christopher	University of Colorado	Kathleen (KC) Woulfe		1	33	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Age-Associated ECHS1 Loss and Sarcomeric Crotonylation Impair Myofibril Relaxation and Mitochondrial Reserve in Female Hearts
Hoogervorst	Remco	Amsterdam University Medical Center			1	35	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Patient-derived iPSC models of HCM reveal acute and chronic mavacamten responses in 2D iPSC-CMs and 3D engineered heart tissues
Hoover Browne	Catherine (Katie)	University of Arizona	Henk Granzier		2	120	Other Topics in Myofilament Biology	Investigating the Physiological Role of the Interaction Between Titin Domains A168-170 and the E3 ligase MuRF1
Irving	Thomas	Illinois Institute of Technology			2	26	Myofilament Structure and Function	New Capabilities of the MuscleX Data Reduction Package for Muscle Diffraction
Irving	Malcolm	King's College London			2	28	Myofilament Structure and Function	Structural changes in the thick and thin filaments during slow stretch of mammalian skeletal muscle
Jani	Vinay	University of Washington	Farid Moussavi-Harami		1	121	Regulation and Modulation of Contraction	Investigation of the Effects of Troponin I Acetylation on Thin Filament Activation with Molecular Dynamics
Janssens	Johannes	Cedars-Sinai Medical Center	Jennifer Van Eyk	X	2	30	Myofilament Structure and Function	Novel Chemical Protein Foot-Printing Mass Spectrometry Methodology for Identification of Thin Filament Structural Differences in Ca2+ Free and Activated States
Jezek	Filip	University of Michigan			2	122	Other Topics in Myofilament Biology	A Mechanistic Cross-Bridge Model of Cardiac Muscle Mechanics Under Energetic Stress
Kampourakis	Thomas	University of Kentucky			1	123	Regulation and Modulation of Contraction	Phase separation controls cardiac myosin light chain kinase activity
Kang	Elaine	University of Arizona	Robbert van der Pijl		2	32	Myofilament Structure and Function	Titin’s N2A Region Impacts Cardiomyocyte Size and Cardiac Remodeling
Kanwal	Aditya	The Jackson Laboratory	J. Travis Hinson		1	37	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Establishing a “poison-peptide” induced murine model of truncated Titin-associated dilated cardiomyopathy
Karpicheva	Olga	Boston University Chobanian & Avedisian School of Medicine			2	38	Myofilament Structure and Function	Quantifying the Structural Basis of Thin Filament Cooperativity: The Effect of Single Myosin Heads on the Position of Tropomyosin on Actin
Kekenes-Huskey	Peter	Loyola University Chicago			1	125	Regulation and Modulation of Contraction	A mechanism for how unstructured regions in the myofilament protein ABLIM1 impact cardiac contractility

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Kim	Taekyeong	The Jackson Laboratory	J. Travis Hinson		1	39	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Base Editing of an Upstream Open Reading Frame in *TTN* to Study Its Regulatory Mechanisms and Potential as a Therapy in DCM
Kim	Andrew	University of Arizona	Jil Tardiff		1	41	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Structural and Functional Impact of the HCM-linked D219N Tropomyosin Mutation
Kolb	Justin	University of Arizona			2	34	Myofilament Structure and Function	Z-disk thickness regulation is independent of titin and nebulin Z-repeats
Kostyukova	Alla	Washington State University			2	36	Myofilament Structure and Function	Lmod2 Orchestrates Actin-Profilin Dynamics to Control Thin Filament Pointed-end Polymerization
Laitila	Jenni	Folkhälsan Research Center			2	84	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Assessing two novel nemaline myopathy mouse models for future therapeutic studies targeting myosin dysfunction
Landim-Vieira	Maicon	Illinois Institute of Technology			1	43	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Danicamtiv Partially Improves Cardiac Contractility in Human Cardiomyopathic Septal Myocardium
Legere	Nicholas	University of Connecticut Health Center	J. Travis Hinson		1	45	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Engineering the Endogenous Cardiac Stress Response using CRISPR-Activation Technology to Create Heart Failure Resilience
Lehman	Sarah	Edgewise Therapeutics			1	47	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Porcine Cardiac Tissue Exhibits Chamber-Specific Enzymatic and Mechanical Profiles Similar to Human Hearts
Lynn	Melissa	University of Arizona		X	1	49	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Intrinsic Modifier Effect of Fetal-cTnT on Disease Progression in Sarcomeric Cardiomyopathies
Ma	Wen	University of Vermont			1	127	Regulation and Modulation of Contraction	Dynamic Allosteric Regulation of Myosin by Cardiomyopathy Mutations and Small-Molecule Modulators
Mariano	Jennifer	University of Arizona	Henk Granzier		2	86	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Pulsatile Myofilament Activity in Myotrem Myopathy Associated with Myogenic Tremor
McAllister	Christopher	University of Pennsylvania	Sharlene Day		1	51	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Cardiac specific AAVs improve heart function in a novel mouse model of hypertrophic cardiomyopathy
McIntyre	Brenna	University of Arizona	Sakthivel Sadayappan		2	88	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Alternative Splicing of *MYBPC1* in Healthy and Diseased Skeletal Muscle
Mijailovich	Srboljub	FilamenTech, Inc.			2	40	Myofilament Structure and Function	In Silico Prediction of Mutation Effects and Drug Efficacy on Cardiac Muscle Function
Minton	Austin	University of Kentucky	Kenneth Campbell	X	1	53	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	TTN Truncating Variants Associate with Increased Passive Stiffness and Regional Fibrosis in Human Non-Ischemic Cardiomyopathy
Mohran	Rhyaan	University of Arizona	Jil Tardiff		2	42	Myofilament Structure and Function	Modeling the effects of cTnI Ser23/24 phosphorylation and the cTnI-R145W Mutation
Moore	Jeffrey	University of Massachusetts Lowell			1	55	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Disruption of Tropomyosin–Troponin T Interactions at Tropomyosin Residue Y261 Alters Thin Filament Cooperativity and Calcium Sensitivity

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Nandwani	Neha	Stanford University	James Spudich		1	57	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Mutation-Dependent Responses to Cardiac Myosin Inhibitors in Hypertrophic Cardiomyopathy
Nelson	Shane	University of Vermont			1	129	Regulation and Modulation of Contraction	Seeing is believing at the single molecule level: Are myosin's IHM and SRX states inextricably linked?
Nieto Morales	Paula	Florida State University	Jose Pinto		1	59	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Impact of a *TNNC1* missense variant on cardiac myofilament function in DCM
Nishikawa	Kiisa	Northern Arizona University			2	44	Myofilament Structure and Function	Titin is a tunable viscoelastic transmission in muscle sarcomeres that regulates energy storage and dissipation
Ostap	E. Michael	University of Pennsylvania			1	61	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Paradoxical Features of Hypertrophic Cardiomyopathy Mutants are Resolved by Single Molecule Optical Trap Studies on Cardiac Actomyosin
Padron	Raul	UMass Chan Medical School			1	63	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Thick Filament Molecular Interfaces Play a Critical Role in the Pathogenesis of Hypertrophic Cardiomyopathy
Pappas	Christopher	University of Arizona		X	1	65	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Leiomodin 2 Mutation-Induced mRNA Decay and Protein Loss Can Be Reversed by Steric-Blocking Oligonucleotides
Pearson	James	National Cerebral and Cardiovascular Center Research Institute			2	104	Myofilament-based Mechano-sensing	Transmural Changes in In Vivo Myosin Motor Dynamics in a Murine Model of Truncated cMyBP-C Hypertrophic Cardiomyopathy
Phan	Trisha	University of Pennsylvania	Sharlene Day		1	67	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Sarcomeric Human Cardiomyopathy Registry data reveals differences in LVEFs and adverse outcome rates in patients with truncating vs non-truncating variants in *MYBPC3*
Plenge	Lucy	University of Pennsylvania	Sharlene Day		2	124	Other Topics in Myofilament Biology	BAG3 interacts with RNA binding proteins to mediate protein turnover in the sarcomere
Powers	Joe	University of Washington		X	1	69	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Deletion of the Z-disk protein Filamin C dysregulates sarcomere length-dependent myofilament structure in resting cardiac muscle
Prodanovic	Momcilo	FilamenTech, Inc. and University of Kragujevac			2	46	Myofilament Structure and Function	Predicted X-Ray Fiber Diffraction Patterns Reveal Heterogeneous Force Distribution in Skeletal and Cardiac Muscle Actin Filaments
Rawish	Elias	University Hospital Schleswig-Holstein	Sakthivel Sadayappan & Ingo Eitel		2	126	Other Topics in Myofilament Biology	Neutrophils Drive Myocardial Dysfunction in Takotsubo Syndrome
Rhodehamel	Marcus	Johns Hopkins University	Mark Ranek & David Kass		1	71	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Left Ventricular Myocyte Structure and Mechanics in Transthyretin Amyloidosis Cardiomyopathy
Risi	Cristina	Old Dominion University			2	48	Myofilament Structure and Function	The Structure of the Native Cardiac Crossbridge in the Rigor State
Rzewnicki	Stephanie	University of Arizona	Sakthivel Sadayappan		1	73	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Myosin S2 in Cardiac Contractility and Hypertrophic Cardiomyopathy Pathogenesis
Sadler	Rachel	University of Arizona	Samantha Harris		2	50	Myofilament Structure and Function	A Gain-of-Function Missense Mutation in Cardiac Myosin Binding Protein-C's M-Domain Promotes the ON State of Myosin Heads in Passive Cardiac Muscle

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Sarparanta	Jaakko	Folkhälsan Research Center			2	92	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Myotube models for studies on C-terminal titin variants
Sauter	Daniel	Sophion Bioscience			1	75	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Functional Maturation and Pharmacological Modulation of Cardiac Contractility in High-Throughput Engineered Heart Tissues
Seffrood	Morgan	University of Arizona	Brett Colson		2	52	Myofilament Structure and Function	Structural Dynamics of Slow Skeletal MyBP-C N-terminal Domains: Effects of Phosphorylation and Splicing in the Pro/Ala-Rich Linker
Shekhar	Shashank	Emory University			2	54	Myofilament Structure and Function	Leiomodin 2 is a processive pointed-end elongator of actin filaments
Sherer	Laura	University of Chicago	Jonathan Kirk	X	1	77	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Characterization of a Stress-Induced BAG3 Cleavage Product in Heart Failure
Smith	Jonathon	National Institutes of Health	Brian Glancy	X	2	56	Myofilament Structure and Function	Interrogation of Skeletal Muscle Contractile Networks with Expansion Microscopy
Smith	John	University of Arizona			2	58	Myofilament Structure and Function	Mice with an expanded Titin C-zone
Solis	Christopher	Florida State University			1	131	Regulation and Modulation of Contraction	Desensitization of the Cardiac Troponin Complex by TnI Phosphorylation and Epigallocatechin-3-Gallate
Somavarapu	Arun Kumar	UMass Chan Medical School			1	79	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Cryo-EM Reveals How Cardiac Drugs (Mavacamten, Omecamtiv Mecarbil), and Cardiomyopathy Mutation (E525K) Modulate the Myosin Interacting-Heads Motif
Song	Taejeong	University of Arizona			2	60	Myofilament Structure and Function	sMyBP-C Is Essential for Tetanic Force Generation by Promoting and Stabilizing Cross-Bridge Formation
Spring	Jessica	University of Arizona	Taejong Song		2	94	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Characterization of Skeletal MyBP-C Expressions in Health and Diseased Muscles
Stahr	Nicholas	University of Arizona	Carl Tong		1	113	Regulation and Modulation of Contraction	Modeling Cooperative Cross-Bridge Dynamics in Beating Heart
Stephanie	Georgina	University of Michigan	Alison Vander Roest		2	106	Myofilament-based Mechano-sensing	H251N β-Cardiac Myosin Mutation Alters Cardiomyocyte Cell-Matrix Force Transmission via Focal Adhesion Remodeling In a Stiffness-Dependent Manner
Sur	Juhi V	Max Delbrück Center for Molecular Medicine		X	2	102	Myofilament-based Mechano-sensing	Titin M-band mechanosensing coordinates uterine peristalsis and embryo implantation
Tanner	Bert	Washington State University			2	108	Myofilament-based Mechano-sensing	Effects of omecamtiv mecarbil on cross-bridge kinetics and power output in rat skeletal muscle fibers
Taylor	Kenneth	Florida State University			2	62	Myofilament Structure and Function	Insect Indirect Flight Muscle and Vertebrate Striated Muscle – Two Solutions to Thick Filament Activation and Endothermy
Thuma	Jenna	Ohio State University	Jonathan Davis		2	128	Other Topics in Myofilament Biology	Troponin Enhanceropathies: A Novel Role for the Troponin Genes

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Ulker	Ali	The Ohio State University	Jonathan Davis		1	81	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	The D73N Troponin C Mouse: A Translational Model of Dilated Cardiomyopathy
van Brakel	Fleur	Amsterdam University Medical Center			2	96	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	The effect of NIV on the respiratory muscle pump in end-stage COPD
Van Den Berg	Marloes	University of Arizona	Coen Ottenheijm		2	90	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Mechanical Ventilation-Induced Diaphragm Weakness and Thick Filament Structural Alterations Persist Following Spontaneous Breathing
Van Den Berg	Marloes	University of Arizona			2	98	Myofilament-based Biology, Diseases and Therapeutics- Skeletal muscle	Preserved Baseline Diaphragm Function with Impaired Contractile Reserve Under Stress in HFpEF
van der Pijl	Robbert	University of Arizona	Henk Granzier		2	64	Myofilament Structure and Function	Thin Filament–MARP1–N2A Tethering Modulates Titin-Based Mechanics and Links to Contractile Dysfunction
van der Pijl	Robbert	University of Arizona	Henk Granzier		2	66	Myofilament Structure and Function	Loss of Novex3 Titin Alters Muscle Function and Reveals a Link Between Sarcomere Organization and Metabolic Regulation
Vander Roest	Alison	University of Michigan			1	83	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Myosin Mutations and Purine Metabolism Impact Contractile Function, Cytoskeletal Structure, and Mitochondrial Function of Human iPSC-derived Cardiomyocytes
Vasquez	Catherine	University of Arizona	Jil Tardiff		1	85	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Small Molecule Z06 Targets Tropomyosin Overlap Flexibility and Improves Systolic Function in a Dilated Cardiomyopathy Mouse Model
Wasley	Tristan	University of Washington	Michael Regnier		2	68	Myofilament Structure and Function	Investigating Cardiac Myosin Binding Protein-C-Mediated Thick Filament Regulation Using In Silico and In Vitro Methods
Watson	Gabriel	King's College London	Elisabetta Brunello		1	133	Regulation and Modulation of Contraction	Sarcomere Length-Dependence of the Structural Changes in the Thick Filament of Demembranated Myocardial Slices from the Rabbit Heart
Yengo	Christopher	Pennsylvania State University			2	70	Myofilament Structure and Function	Structural Dynamics of the Autoinhibited Interacting Heads Motif in Human Beta-Cardiac Myosin
Yoder	Micah	University of Chicago	Jonathan Kirk		2	130	Other Topics in Myofilament Biology	The Sweet Taste Receptor Regulates Cardiomyocyte Contractility and May Regulate Cardiomyocyte Bioenergetics
Yurumez	Eslem	Amsterdam University Medical Center			1	87	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Understanding the Mechano-Signaling Role of the Z-disc in the Pathogenesis of Hypertrophic Cardiomyopathy
Zanella	Helena	The Ohio State University	Brandon Biesiadecki		2	72	Myofilament Structure and Function	Cardiac troponin I tyrosine phosphorylation differentially regulates myofilament function
Zanetti	Michele	Yale University	Stuart Campbell	X	1	89	Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle	Cardiac Hypercontractility as a Causal Mechanism of Congenital Heart Disease

Poster #1

The interfibrillar mitochondrial metabolome in aged female hearts leads to a unique microenvironment and alters acylation of sarcomeric proteins.

Mackenzie J Amrine[1,3], Christopher W Hoffer[1,3], Lori A Walker[1], Timothy McKinsey[1], Anastacia M Garcia[1,2,3], Kathleen C Woulfe[1,3*]

[1] Department of Medicine, Division of Cardiology, University of Colorado, Anschutz Medical Campus, Aurora, CO

[2] Division of Cardiology, Department of Pediatrics, Children's Hospital of Colorado, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA

[3] Integrated Physiology Program, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA

By 2030, it is estimated that more than 50% of Americans will suffer from heart failure as a result of diastolic dysfunction. Notably, there is a dramatic increase in the incidence of diastolic dysfunction in postmenopausal women; however, very little is known about mechanisms underlying age-specific changes that occur in the female heart. We have found that sarcomeric proteins isolated from the hearts of post-menopausal women relax significantly slower compared to the hearts of pre-menopausal women. To better understand changes that may be occurring in the myofibrillar proteome, we measured myofibril enriched protein expression by unbiased mass spectrometry. From this data, we found evidence of specific interfibrillar mitochondrial dysfunction and abnormal fatty acid metabolism. Based on this, **we hypothesize that age specific changes in the microenvironment between sarcomeres and interfibrillar mitochondrial lead to altered metabolomics and therefore altered acylation landscape on sarcomeric proteins modifying function**. We compared the global metabolome of non-failing ventricles from 20–40-year-old females and males, and >60-year-old males and females with the localized interfibrillar metabolome from the same patients. This revealed dysfunction in long-chain fatty acids and acyl-carnitines uniquely upregulated in only the myofibril-enriched metabolome from heart of females over 60 years of age. To determine the impact of these unique elevated acyl-carnitines on aged female cardiomyocyte dynamics and sarcomeric function, we treated adult female rat ventricular cardiomyocytes with 10μM Arachidonoyl-L-carnitine (AA-Car) for 24 hours. Cells treated with AA-car showed slower relengthening dynamics and prolonged sarcomeric relaxation. These data suggest the altered metabolomic microenvironment of interfibrillar mitochondria in aged females leads to key functional changes in the sarcomere.

Modeling myosin's departure from the IHM with molecular simulation

Matthew C. Childers, Michael A. Geeves, and Michael Regnier

Myosins are essential molecular motors that drive muscle contraction and regulate cardiac output. A key mechanism for tuning myosin activity is the formation of the *interacting heads motif* (IHM), an autoinhibited conformation that sequesters myosin heads and reduces ATPase activity. Dysregulation of IHM stability is implicated in cardiomyopathies, yet the molecular mechanisms governing transitions out of the IHM state remain poorly understood due to the size and complexity of the system. We used long-timescale molecular dynamics simulations on Anton 3 to model the dissociation of protein-protein interfaces that stabilize the cardiac β-myosin IHM. We performed simulations on two simplified constructs that isolate the principal stabilizing interactions—the blocked head–free head interface and the blocked head–S2 interface—we introduced physiologically relevant destabilizing factors including disease-causing mutations (R403Q, K450E), regulatory light chain phosphorylation, high [KCl] and the ADP analogue dADP. This design enabled us to probe conformational heterogeneity and capture the sequence of structural events leading to IHM departure. Our simulations provide unprecedented molecular detail of IHM exit, revealing how specific perturbations alter protein-protein interfaces and shift the balance between inactive and active states. These findings advance the mechanistic understanding of myosin regulation, highlight structural features that can be targeted by small molecules, and establish a framework for modeling transitions into and out of the IHM conformation.

Rbm24 Mediated Alternate Splicing of *MYBPC3* in Hypertrophic Cardiomyopathy

Kalyani Ananthamohan[1], Pooneh Navbavizadeh[2], Akhil Baby[1], Taejeong Song[1], James W. McNamara[2], Jonathan Kirk[3], Mohammad Bohlooly[4], Katja Madeyski-Bengtson[4], Ralph Knöll[4], and Sakthivel Sadayappan[1]

1. Department of Cellular and Molecular Medicine, Sarver Heart Center, University of Arizona College of Medicine, Tucson, AZ, USA.
2. Department of Internal Medicine, Division of Cardiovascular Health and Disease, University of Cincinnati College of Medicine, Cincinnati, OH, USA.
3. Department of Cell and Molecular Physiology, Stritch School of Medicine, Loyola University Chicago, Chicago, IL, USA.
4. Cardiovascular, Renal and Metabolism, R&D Biopharmaceuticals, AstraZeneca, Gothenburg, Sweden.

Background: Mutations in cardiac myosin binding protein-C (*MYBPC3*) are a major cause of hypertrophic cardiomyopathy (HCM). Despite the large number of splice-disrupting mutations identified in *MYBPC3*, the roles of alternative splicing and splicing factors in *MYBPC3*-mediated HCM remain underexplored. The primary objective of this study was to systematically investigate splice factors and alternative splicing mechanisms regulating *MYBPC3* expression and function. Our working hypothesis is that upregulation of RNA-binding motif protein 24 (*Rbm24*) enhances *MYBPC3* alternative splicing, thereby exacerbating the development and progression of HCM.
Methods and Results: To test our hypothesis, we utilized a humanized mouse model and human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) carrying a 25-bp deletion in intron 32 of *MYBPC3* (*MYBPC3*$^{\Delta 25bp}$), a variant inherited by over 100 million individuals and associated with HCM. The *MYBPC3*$^{\Delta 25bp}$ variant is located at the branch point of intron 32 and disrupts the splice branch point and polypyrimidine tract. In silico analyses predicted both gain and loss of splice donor/acceptor sites, as well as alterations in putative splice factor–binding motifs associated with *MYBPC3*$^{\Delta 25bp}$. Results from exon-trap assays, hiPSC-CMs harboring *MYBPC3*$^{\Delta 25bp}$, and a novel humanized *Mybpc3*$^{\Delta 25bp}$ mouse model demonstrated that this variant induces cryptic splicing involving exon 33 (ΔExon33), accompanied by significantly elevated *RBM24* mRNA expression. Furthermore, *Mybpc3*$^{\Delta 25bp}$ mice exhibited impaired contractile function compared with wild-type controls. Upon aging these mice displayed markedly increased ΔExon33 splice transcripts, where partial functional recovery was observed following treatment with ΔExon33 antisense oligonucleotides (ASOs). To determine whether *RBM24* upregulation alone is sufficient to exacerbate aberrant splicing, MyoAAV4-Rbm24 was administered to homozygous *Mybpc3*$^{\Delta 25bp}$ mice. This intervention led to a significant increase in ΔExon33 splice transcript expression, further supporting a causal role for Rbm24 in modulating *MYBPC*3 alternative splicing and HCM pathogenesis.
Conclusions: Taken together, these findings highlight the importance of *MYBPC3* alternative splicing in the pathogenesis of HCM, potentially mediated by Rbm24 upregulation. Therapeutic strategies that modulate Rbm24 expression or activity may represent a promising approach to reduce aberrant *MYBPC3* alternative splicing and improve cardiac outcomes in HCM.

Structural Polymorphism of the Cardiac Actin Y166C as a Molecular Basis for Hypertrophic Cardiomyopathy

Hsiang-Ling Huang[1], Andrejus Suchenko[2], Mohan K. Balasubramanian[2], Sarah M. Heissler[1], Krishna Chinthalapudi[1]

[1]Department of Physiology and Cell Biology, Dorothy M. Davis Heart and Lung Research Institute, College of Medicine, The Ohio State University; Columbus, Ohio, USA.

[2]Centre for Mechanochemical Cell Biology and Warwick Medical School, Division of Biomedical Sciences, Coventry, United Kingdom.

Cardiac muscle contraction depends on coordinated interactions between thin-filament α-actin and thick-filament myosin within sarcomeres. Mutations in cardiac α-actin cause hypertrophic cardiomyopathy (HCM), a disease characterized by ventricular wall thickening and risk of heart failure. The Y166C pathogenic mutation is strongly associated with HCM, but its molecular mechanism remains unclear. Here, we present cryo-EM structures of wild-type (WT) and Y166C cardiac actin filaments. The Y166C substitution at the longitudinal interprotomer interface induces subtle helical rearrangements but substantially weakens longitudinal and lateral contacts, increasing filament flexibility. The mutation also narrows the nucleotide-binding pocket and opens the phosphate-release “back door.” We identify an allosteric network linking residue 166 to the C-terminus and nucleotide-binding site, coupled to a conformation resembling myosin-bound actin, consistent with enhanced myosin affinity from free-energy calculations. 3D variability analysis reveals structural heterogeneity and varied flexibility in Y166C filaments, supported by dynamic cross-correlation maps showing coordinated motions along the allosteric pathway. Together, our results demonstrate that Y166C remodels both the static architecture and dynamic behavior of cardiac actin filaments, altering actin-myosin interaction landscapes that may underlie HCM pathogenesis.

Aging exacerbates contractile dysfunction in a mouse model of atrial myopathy

Cizauskas HE[1], Bui T[1], Cao Q[1], Edassery S[1], Barefield DY[1]
[1]Loyola University Chicago, Department of Cell and Molecular Physiology

Atrial Fibrillation (AFib) is a common arrhythmia associated with atrial myopathy, with aging as its strongest risk factor. Preclinical AFib models rarely incorporate aging, nor do they assess atrial sarcomere function. We hypothesize that interplay between aging and atrial myopathy exacerbates AFib pathophysiology.

A mouse model of atrial myopathy with inducible AFib via 3-week angiotensin-II (AngII) infusions were used on young (4-month) and aged (18-month) mice. ECG recordings showed decreased P-wave duration in young AngII and aged saline mice (18.15±0.75;18.5±0.63 ns) compared to young saline mice (14.9±0.57 ns). AngII had significantly slowed AV node conduction only in the aged mice, with prolonged P-R durations (60.35±3.5 v. 52.93±2.54 ns). Echocardiography showed LVPW;d hypertrophy with AngII v. saline (1.03 ns±0.06 v. 0.78±0.03 mm), which was more severe in aged AngII v. saline (1.33±0.05 v. 0.86±0.04 mm). Eccentricity was assessed by normalization LV wall thickness to total LV diameter, showing significant hypertrophy with AngII treatments (young: 0.38±0.012 v. 0.31±0.006; old: 0.41±0.011 v. 0.33±0.008), indicating concentric cardiac enlargement with age. LV fractional shortening was significantly increased with AngII only in young mice (35.8±1.5 v. 28.6±1.85 %). Diastolic dysfunction was decreased by AngII only in aged mice including isovolumic relaxation time (12.97±1.95 v. 19.78±1.22 ms) and E/e' ratio (-65.28±7.69 v. -69±4.54). These data indicate age-dependent remodeling of the left ventricle.

Atrial myopathy was assessed using B-mode echocardiography of a left lateral long-axis plane, visualizing the complete 2D coronal atrioventricular plane. Aged AngII mice showed left atrial enlargement compared to aged saline (10.07±0.76 v. 7.48±0.25 μm^2), reduced left atrial ejection fraction (21.01±2.56 v. 43.41±1.19 %), decreased reservoir and contractile strain (reservoir: 35.5±3.93 v. 61.54±8.87 %; contractile: 37.1±5.18 v. 56.15±4.42 %), and frequent occurrence of atrial-ventricular desynchrony. Mass spectrometry revealed age- and AngII-dependent proteomics changes in major known age- and AFib-related pathways. Additionally, increases in *Tpm3* and *Myl2* reveal sarcomere alterations in AFib pathological remodeling in the aged heart.

Overall, we provide the first characterization of atrial dysfunction in the aged, AFib mouse heart and identified myofilament proteins as potential mediators of AFib in the aged heart.

Genetically edited insect flight muscle: a novel resource for recombinant sarcomeric actin production and *in vivo* investigation

Kripa Chitre, Aditi Madan, Indira Paddibhatla, Meaghan Barry, William Schimdt, Meera C. Vishwanathan, Skylar M. L. Bodt, Axel J. Fenwick, D. Brian Foster, Anthony Cammarato

[1]Department of Medicine, Division of Cardiology, Johns Hopkins University, 720 Rutland Avenue, Baltimore, MD 21205, USA.

[2]Department of Physiology, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205, USA.

Actin forms many cytostructural elements, including the backbone of striated muscle thin filaments. Our mechanistic understanding of most actin-dependent processes remains limited, partly due to the challenges with recombinant actin expression. Historically, bacteria have failed to generate fully-functional actin, however baculovirus-insect cell expression systems have proven successful. Here, we created a tissue-based tool for recombinant sarcomeric actin production, for hypothesis testing and for resolving *ex vivo* molecular properties and *in vivo* cellular effects of unique variants. Specifically, we engineered *Drosophila* that can express untagged “designer” or pathological actins exclusively in indirect flight muscles (IFMs). *Act88F* encodes sarcomeric IFM actin. Attempts to rescue IFM function (i.e. flight) in *Act88F*-nulls, by inserting transgenic *Act88F* (*Act88F*$^{TG\text{-}WT}$) ectopically throughout the genome have failed, possibly due to ineffective cis/trans regulation of *Act88F*$^{TG\text{-}WT}$ expression. To circumvent this potential issue, we used CRISPR-editing and introduced an *attP* landing site directly into *Act88F*, to disrupt the endogenous gene and serve as a target for site-specific transgene insertion. *attP*-mediated disruption of *Act88F* yielded flightlessness and actin-free IFMs. Transformants expressing *GFP* or *Act88F*$^{TG\text{-}WT}$, from the endogenous *Act88F* integration locus, demonstrated robust IFM-specific protein production and restoration of flight, respectively. Having established the expression system, we created *Drosophila* mutants to test the roles of specific actin residues in tropomyosin/myosin-binding and nemaline myopathy pathogenesis. Both *Act88F*$^{TG\text{-}R147Q}$ and *Act88F* $^{TG\text{-}F352S}$ triggered IFM hypercontraction and impaired flight. The former exhibited a dose dependent auto-destruction, which was partially rescued by abrogating acto-myosin interactions *in vivo*, but displayed an unexpected reduction in sliding velocities *in vitro* compared to WT counterparts. Moreover, we are currently performing proteomic analysis of Act88F vs. Act88F$^{TG\text{-}WT}$ to ensure proper post-translational processing of the recombinant protein. Finally, we determined whether mammalian actin could be generated and observed high-level expression of human α-cardiac actin (ACTC1), which behaved indistinguishably from human ACTC1 isolated from non-failing left ventricular biopsies in unregulated and regulated motility assays. Overall, our novel fly line permits transgenic actin expression in mature muscle fibers that are themselves amenable to structural and functional analyses, and serves as a cost-effective, perpetual source of ample protein for biophysical experimentation.

Poster #7

Phosphorylation of TnI-T181:

Redefining HFpEF, model organisms, and potential therapeutic targets

Meaghan E. Barry [1], Marcus Rhodehamel [1], Vivek Jani [1], Axel Fenwick [1], Romi Castillo [2], Maria T. Giannakopoulos [1], Jill Tardif [2], David A. Kass [1], and Anthony Cammarato [1]

[1] Division of Cardiology, Department of Medicine, Johns Hopkins University, Baltimore, MD

[2] Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ

An estimated 6.7 million adults in the US have clinical heart failure, with over half having heart failure with preserved ejection fraction (HFpEF). With HFpEF, the EF is maintained and some degree of diastolic dysfunction with elevated left ventricular diastolic pressures are common. Currently, over 80% of patients diagnosed with HFpEF have a body mass index (BMI) >30, classified as obese. Highlighting the complexity of this syndrome, recent studies reveal cardiomyocytes and myofibrils from severely obese HFpEF patients have depressed activated sarcomere mechanics versus less obese HFpEF and non-failing controls. Phosphoproteomics uncovered post-translational modifications of the thin filament from myocardial biopsies, with a particular modification on troponin I (TnI) at threonine 181 (T181) being hyperphosphorylated. Moreover, p/total T181 directly correlated with BMI in HF but not non-failing patients. Multiple sequence alignment analysis reveals high conservation of T181, except for an alternative isoleucine in the Muridae family that includes the highly used model organisms: mouse and rat. The goal of this study was to (1) characterize the functional effect of phosphorylation at T181, (2) identify a viable model organism to study the physiological effects of HF in the context of this PTM, and (3) identify the kinase responsible for T181 phosphorylation.

Recombinant human Tn constructs with the phosphomimetic, aspartic acid substitution (T181D), were exchanged into cardiomyocytes as well as reconstituted with actin and tropomyosin for *in vitro* motility (IVM) assays, revealing a reduction in maximum tension at saturating calcium (T_{max}) and increased calcium sensitivity (WT: T_{max} = 24.39 mN/mm^2 vs T181D:15.03 mN/mm^2 , P<0.001; WT: EC_{50}= 3.30 uM vs T181D EC_{50}= 2.26 uM, P<0.001) (IVM, WT V_{max} = 3.99 um/sec vs T181D V_{max} = 1.92 um/sec, P<0.001; WT pCa_{50}= 6.16 vs T181D pCa_{50}= 7.19, P=0.03). Cardiomyocyte mechanics in Guinea Pig models of HF (transverse aortic constriction and isoproterenol), a mammal with TnI-T181, exhibits depressed T_{max} as in humans. We previously reported no changes in the common mouse and rat HFpEF models. Moving forward, the GP model can be used to study WT and phosphomimetic recombinant troponin, testing impacts in vivo as well. Through these models, we hope to better characterize the functionality of this modification, identify the responsible kinase(s), and lay the foundation for potential targeted therapeutics for severely obese HFpEF.

Comparing Cryo-EM Structures of the Vertebrate Cardiac Muscle Thick Filament

Roger Craig[1], Debabrata Dutta[1], Natalia A. Koubassova[2], Andrey K. Tsaturyan[3], Raúl Padrón[1]

[1]University of Massachusetts Chan Medical School, Worcester, MA, USA; [2]Lomonosov Moscow State University, Moscow, Russia; [3]Tel Aviv University, Tel Aviv, Israel

An atomic model of the vertebrate striated muscle thick filament has been lacking. Three cryo-EM filament structures from vertebrate cardiac muscle have now been reported, revealing myosin heads in interacting-heads motifs (IHMs) on the thick filament surface, and a complex arrangement of myosin tails, titin, and cMyBP-C in the filament backbone. The three studies are complementary, coming from two species (mouse, human), two techniques (cryo-ET and single-particle cryo-EM), and from muscles treated or not with mavacamten. The structures are remarkably similar, agreeing on most elements of molecular organization but differing in stability of the IHMs and the organization of cMyBP-C. The differences are explained by the different techniques and the observation of filaments within, or isolated from, the filament lattice.

The mavacamten-stabilized filaments show IHMs in all three crowns of the 430-Å repeat. These crowns have differently organized IHMs, and their tails follow different paths, forming the filament core, a surrounding shell, and a surface layer, which functions to dock cMyBP-C. Two titin molecules run along each sector of the 3-fold symmetric filament, positioning the myosin tails. In the isolated filament, cMyBP-C runs longitudinally, while in the filament lattice, the molecule bends out towards the adjacent thin filament. The absence of actin in the isolated preparation explains the difference in orientation of cMyBP-C. cMyBP-C C-terminal domains interact with the IHMs, enhancing SRX in the C-zone. Remarkably, the IHMs in one crown of the isolated filament are azimuthally displaced ~70 Å from the same IHM of filaments in the lattice, suggesting a direct influence of actin on this IHM, and supporting its proposed ‘sentinel’ role in detecting thin filament activation.

Thick filaments without mavacamten show similar results, except for a striking disorder of most of the IHMs. This most likely results from the low temperature (4°C) used to prepare the specimens rather than from the absence of mavacamten: X-ray diffraction shows major disordering of myosin helices at low temperature. X-ray patterns at room temperature show only minor enhancement of ordering with mavacamten, consistent with well-ordered IHMs under non-drug conditions, similar to the mavacamten cryo-EM results.

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The Impact of Myosin Modulators on Myofilament Dysfunction in Obese Heart Failure with Preserved Ejection Fraction

Skylar M. L. Bodt, Axel J. Fenwick, David A. Kass, Anthony Cammarato

Division of Cardiology, Department of Medicine, Johns Hopkins University, Baltimore, MD

Heart failure with preserved ejection fraction (HFpEF) is a multiorgan disease, where the heart maintains a normal range EF, but often exhibits objective diastolic dysfunction. Accounting for over half of heart failure cases, its prevalence is increasing alongside metabolic and hypertensive comorbidities, giving rise to distinct subphenotypes. These include patients with less obesity but more prominent hypertension/hypertrophy, those with severe obesity but less hypertrophy and hypertension, and a group exhibiting both features. Isolated cardiomyocyte studies have shown subgroup-specific differences in contractile performance and elevated diastolic tension. Resolving the rapid kinetics of activation and relaxation is essential for defining the molecular basis of HFpEF, parameters best measured in single myofibrils. Such fibrils were isolated from human right ventricular biopsies and mounted between a piezoelectric length controller and a cantilever-based force transducer capable of detecting nanonewton-scale tension changes. Rapid switching between low-calcium (pCa 8) and high-calcium (pCa 4) solutions via a multi-barreled pipette enabled sub-millisecond resolution of activation and relaxation kinetics. Using this custom-built system, we observed reduced maximal tension in myofibrils from highly obese HFpEF patients and impaired relaxation across all subgroups, implying myofilament-level dysfunction contributes to diastolic impairment. These findings are especially relevant considering the EMBARK-HFpEF clinical trial investigating the myosin inhibitor Mavacamten as a viable treatment for HFpEF. In preliminary data, we find Mavacamten accelerates relaxation but also markedly suppresses active tension in obese HFpEF. Thus, while negative myotropes may confer some benefit on diastole, patients with severe obesity should be very carefully considered and are unlikely candidates for this treatment. We are actively testing other modulators, such as myosin activator Danicamtiv, that may improve active tension without compromising diastolic tension in HFpEF with severe obesity.

In Silico Analysis of Conformational Dynamics of the Alpha-Actinin-2 Actin-Binding Domain in Response to Hypertrophic Cardiomyopathy Mutations

Rhiannon Crawford and Christopher Solís

[1] Department of Health, Nutrition, and Food Sciences, Florida State University, Tallahassee, FL, 32306

***Email:** csolis@fsu.edu

Abstract

The goal is to understand the mechanism by which α-actinin-2 (ACTN2) is involved in the assembly of sarcomeres in striated muscles. ACTN2 is a key protein in the Z-disc at the actin binding domain (ABD) of sarcomeres, helping maintain sarcomere structure and anchor actin filaments. ACTN2 mutations in patients have been associated to cause hypertrophic cardiomyopathy (HCM) and distal myopathy (DM). However, the mechanism of action of these mutations on the ABD are still unknown. To elucidate the conformational changes of the ACTN2 ABD in its closed state, structural modeling of some common HCM mutation sites was completed using AlphaFold3 at the HCM-mutations Ala119Thr, Met228Thr, and Thr247Met; and the DM Leu131Pro mutation. The coulombic charges and the distances between the center of mass between the CH1 and CH2 domains were measured for WT and all the mutants. NetPhos 3.1 was also used to compare any different kinases present in the various mutations. AlphaFold3 found significant structural differences in the mean center of mass distances for all mutations except Ala119Thr, with the mutations generating a greater distance between the domains. However, there were no significant differences between normal and mutated ABD for the coulombic charges. From NetPhos 3.1, potential new kinase binding sites in the mutant sequences ACTN2 were identified. These results suggest that mutations in ACTN2 may affect the structure of the ABD, promoting a more open conformation, possibly allowing further interactions with actin.

ACTC1 A319_L320*del*: A Model Variant to Characterize Subdomain 3 Thin Filament Dysregulation in Cardiomyopathy Development

Vanessa Brudz* & John Dawson

University of Guelph, Department of Molecular and Cellular Biology

Cardiovascular diseases (CVDs) remain the leading cause of global mortality, with cardiomyopathies representing a major genetically driven subset that often culminates in heart failure. Among these, hypertrophic and dilated cardiomyopathies (HCM and DCM) commonly arise from mutations in genes encoding proteins of the sarcomere resulting in dysfunction of the left ventricle. The alpha cardiac actin gene (*actc*1) encodes the predominant actin isoform in cardiac muscle, forming the structural core of thin filaments and regulating contraction through coordinated interactions with myosin for ATP-driven force generation, with these interactions being regulated by troponin and tropomyosin in a Ca^{2+}-dependent manner. Disruption of these interactions through *actc*1 mutations alters filament stability and sarcomere mechanics, contributing to disease.

This study investigates the biochemical and functional consequences of a novel deletion mutation, A319_L320*del* (AL) identified in zebrafish. This mutation results in the removal of key hydrophobic residues within the tropomyosin-binding region of ACTC1 subdomain 3, a site critical for electrostatic interactions that stabilize tropomyosin in the blocked (off) state. We hypothesize that this deletion destabilizes the blocked configuration, reducing the energetic barrier to thin-filament activation, thereby increasing Ca^{2+} sensitivity and impairing relaxation of the left ventricle under diastolic conditions. This is supported by preliminary *in vitro* motility assays which depict a leftward shift in Ca^{2+} activation curves, indicating heightened Ca^{2+} sensitivity and impaired relaxation kinetics, suggesting that AL destabilizes tropomyosin's blocked state, biasing thin filaments toward a constitutively active configuration.

Ongoing single-molecule TIRF microscopy studies aim to resolve how AL alters tropomyosin binding cooperativity and overall filament stability. Integrating these data with *in silico* molecular modelling and *in vivo* phenotyping in zebrafish will clarify how subdomain 3 perturbations distort actin's allosteric network. In the long term, defining these residue-specific mechanisms may refine personalized therapeutic strategies, enabling molecularly targeted interventions for ACTC1 mutation carriers and guiding genotype-driven approaches to restore thin-filament regulatory balance in cardiomyopathies.

Pathophysiological Significance of Linker and Loop Motifs of Cardiac Myosin Binding Protein-C

Garrett S. Crosby[1], Steph R. Rzewnicki[1], Marloes van den Berg[1], Alexey Dvornikov[2], Patrick Beauchamp[1], Tom Bunch[1], Brent Cernyar[1], Brett A. Colson[1], Samantha P. Harris[2] and Sakthivel Sadayappan[1]

[1] Department of Cellular and Molecular Medicine, Sarver Heart Center,

University of Arizona College of Medicine, Tucson, AZ, 85721

[2] Department of Physiology, University of Arizona, Tucson, AZ 85721

Background: Cardiac myosin binding protein-C (cMyBP-C) contains 11 domains (C0-C10) organized into N-terminal (C0-C2), middle (C3-C7), and C-terminal (C8-C10) regions. The N-terminus regulates contraction via interactions with actin and myosin, while the C-terminus anchors cMyBP-C to the thick filament. The middle domains modulate N-terminal function and harbor mutations associated with hypertrophic cardiomyopathy (HCM). However, the structural and physiological roles of the 11-amino-acid (aa) linker between C4 and C5 (C4−C5L), the 28-aa loop in C5 (C5Loop), and the 17-aa linker between C9 and C10 (C9−C10L) of cMyBP-C remain unknown.

Hypothesis: The C4−C5L, C5Loop, and C9−C10L of cMyBP-C are necessary for maintaining normal cardiac structure and function at the cellular, molecular, and organ levels.

Methods and Results: To test this hypothesis, novel CRISPR-Cas9–mediated knockout mouse models lacking C4−C5L, C5Loop, and C9−C10L were generated to systematically define the roles of these linkers and the loop. Echocardiography analyses revealed that heterozygous (het) ΔC4−C5L mice exhibited diastolic dysfunction at 5 months of age while homozygous (hom) ΔC5 loop mice exhibited no cardiac dysfunction. Het ΔC9−C10L mice exhibit mildly altered diastolic function at 6 months. Hom ΔC9−C10L mice exhibit increased HW/BW ratios, decreased systolic function, increased left ventricular wall thickness, and diastolic dysfunction at 3 months. Initial myofibril ATPase rates from het ΔC4−C5L mice were increased compared to wild-type (WT) myofibrils at saturating calcium (pCa 4), suggesting the linker plays a role in modulating actomyosin interactions. However, ATPase rates in hom ΔC5Loop myofibrils exhibited no difference compared to WT, suggesting that the C5Loop does not modulate actomyosin interactions. Preliminary FRET data suggest that the C4−C5L is critical for cMyBP-C to adopt a bent or folded conformation. In WT C3-C7, FRET probes positioned on domains C4 and C5 report a distance of ~4 nm. In contrast, in C3-C7 lacking the C4−C5L, this distance exceeds the measurable FRET range (>7 nm), indicating that C3-C7 adopts a more extended, linear conformation in the absence of the C4−C5L.

Conclusion: Taken together, results suggest functional roles for both the C4−C5L and the C9−C10L, but the physiological significance of the C5Loop remains unknown.

Poster #13

Biophysical, Biochemical, and Structural Analyses of *Drosophila* Cardiac Myosins and Effects of Dilated Cardiomyopathy Mutations

Jared Rafael T. Camillo[1], Clara L. Neal[1], Morgan I. Mullens[1], William A. Kronert[1], Yuezhi Mao[2], Tom Huxford[2], Sanford I. Bernstein[1]

[1] Department of Biology, Molecular Biology Institute, Heart Institute, San Diego State University, San Diego, CA, 92182 USA

[2] Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182 USA

We previously collaborated to identify and assess the muscle mechanical properties of two major myosin heavy chain isoforms expressed in the *Drosophila* cardiac tube. To this end, “CardM1” and “CardM2” (which differ at the lip of the ATP-binding region, the relay domain, and the converter domain) were transgenically expressed in the indirect flight muscles (IFMs) and tergal depressor of the trochanter (TDT or jump) muscle (PMID: 39799399). Ultrastructural analysis revealed that IFMs and TDT muscle expressing either isoform assemble normal myofibrils; however, flies exhibited impaired jump and flight abilities. Further, mechanical assays of TDT muscle fibers demonstrate that CardM2 produces significantly greater stretch activation (the delayed increase in force generation following muscle stretch) relative to CardM1 and the TDT muscle control at elevated inorganic phosphate concentrations, suggesting a putative role in effectuating myosin-based stretch activation in the heart. We now show that CardM1 and CardM2 exhibit decreased actin-activated ATPase V_{max} values and decreased unloaded *in vitro* actin filament sliding velocities compared to TDT muscle and IFM myosin isoforms. To determine the effects of human dilated cardiomyopathy (DCM) mutations on *Drosophila* cardiac myosins, we introduced R237W or S532P codons into CardM1 and CardM2 transgenes and expressed them in flies via phiC31 integrase-mediated transformation. AlphaFold 3 3D modeling was used to predict the rigor-state actomyosin complexes of CardM1 and CardM2, with energy minimization performed via the application of the ff14SB force field and the General Amber Force Field (GAFF) using Assisted Model Building with Energy Refinement (AMBER). CardM1 and CardM2 are predicted to exhibit differences in the coordination of Loop 1, a region involved in phosphate release, with the lip of the ATP-binding region, suggesting a role for isoform-specific nucleotide affinity in myosin-based stretch activation. Notably, our predictive models suggest that the R237W substitution introduces a hydrophobic interaction within the phosphate tube that correlates with a moderate kink (~10°) in the relay domain, which may have implications for intramolecular communication and for the power stroke. Ongoing efforts include measuring the mechanical, biochemical, and functional capacities of the CardM1 and CardM2 DCM myosins to observe the extent of pathologies induced by these mutations.

Poster #14

Chamber-Specific Responses to Length Dependent Activation in Porcine Myocardium

Jessica Tolley, Sarah Lehman, Mike DuVall, Kat Chmykh, Marcus Henze, Craig Emter, Alan J Russell
Edgewise Therapeutics, Boulder, CO

Pig hearts have often been used as surrogates to assess potential therapeutics in preclinical studies, but few studies have examined chamber-dependent responses to stretch. Length-dependent activation (LDA) is a mechanism that increases the length of the sarcomere, which increases contractile force, calcium sensitivity, and activates cross-bridges from the super-relaxed state (SRX) into the disordered-relaxed state (DRX). This study assessed skinned fibers from the left ventricle (LV) and left atria (LA) of wild-type porcine at short and long sarcomere lengths. LV Force-pCa showed higher calcium sensitivity and cooperativity (5.93 ± 0.02 [P<0.001], 2.91 ± 0.10 [P<0.01]) than LA (5.84 ± 0.01, 2.41 ± 0.09). X-ray diffraction at short showed greater intensities in 1,1/1,0 in LA over LV (0.745 ± 0.05 [P<0.0001], 0.362 ± 0.02) and MLL1 in LV over LA (4.109 ± 0.84 [P<0.0001], 0.193 ± 0.02), indicating more myosin heads are farther away from the thick filament backbone in LA. Passive force showed similar peak force after applying a stretch protocol in the absence of calcium on LA and LV (16.84 mN/mm^2 ± 4.10, 18.10 mN/mm^2 ± 8.5). LDA analysis showed a similar change at stretch in LA/LV fibers in pCa50 (1.57% ± 0.12, 1.45% ± 0.11) and hill slope (0.31 ± 0.07, 0.19 ± 0.07). LV tension increased at pCa6, pCa4.5 (57.44% ± 7.56 [P<.05], 13.28% ± 3.11) to LA (27.23% ± 9.39, 4.87% ± 4.41). At stretch, the %DRX population in LA and LV fibers increased (84.3 ± 4.6 [+13.9%], 75.2 ± 3.6 [+12.8%]), %AUC of LA decreased (85.83% ± 4.03 [P<0.05]). Overall, LA/LV showed similar passive force, stretch differences arose in active force. Both have similar %DRX increase in response to stretch, but atria has more myosin heads in the DRX state which leads to a greater DRX population at stretch, but LDA favors ventricle 2-fold. This suggests that there may be potential differences in recruitability, where structural ON/OFF states may not be directly linked to biochemical SRX/DRX[1]. This data highlights the complexity of the LDA response in the different chambers of the pig heart, and the need for further interrogation.

Consequences of a *TTN* Truncating Mutation on Active and Passive Mechanics of Human Engineered Heart Tissue

Andrés W. Cubero Cruz[1], Emerson Soo Ho[1], Zijian Li[3], Aditya Kanwal[3], Abhijit Takawale[3], J. Travis Hinson[3,4], Stuart G. Campbell[1,2]

1. Biomedical Engineering Department, Yale University, New Haven, CT
2. Department of Cellular and Molecular Physiology, Yale Medical School, New Haven, CT
3. The Jackson Laboratory for Genomic Medicine, Farmington, CT
4. Cardiology Center, University of Connecticut Health Center, Farmington, CT

Background: Dilated cardiomyopathy (DCM) is a genetic cardiac disorder characterized by left ventricular dilation and contractile dysfunction, ultimately leading heart failure. Truncating variants in the titin gene (*TTN*) are among the most common genetic causes of DCM. We sought a better understanding of how TTN truncating variants alter muscle function, and the potential for restoring normal function through adenine base editing (ABE) correction of truncating variants.

Materials and Methods: CRISPR-Cas9 was used to heterozygously introduce a truncating A-band *TTN* mutation into an otherwise healthy human induced pluripotent stem cell (iPSC) line. Edited iPSCs were differentiated into cardiomyocytes and seeded onto engineered heart tissues (EHTs) composed of 6 mm segments of decellularized porcine left ventricular myocardium mounted in custom teflon frames. In addition to the heterozygous truncating *TTN* mutant (TTNtv), EHTs were formed from the isogenic wild-type (WT) line and a line in which ABE had been used to correct the *TTN* truncation (TTNtv-ABE). EHTs were cultured for 21 days before mechanical testing to assess isometric twitch contraction and passive force at various tissue lengths. These data were then used to construct length-dependent activation and passive stress-strain curves.

Results: WT tissues exhibited a robust length-dependent increase in force. In contrast, HET tissues demonstrated a blunted response to stretch, indicating impaired LDA due to titin haploinsufficiency. The gene-edited TTNtv-ABE tissues showed a significant recovery of contractile function, with force values approaching those of WT across all stretch conditions. A mixed-effects model revealed significant main effects for stretch ($p < 0.0001$), genotype ($p = 0.0008$), and their interaction ($p < 0.0001$), confirming both the biological and treatment-dependent variability in force output. Unexpectedly, TTNtv EHTs displayed elevated diastolic force levels and greater tissue stiffness compared with WT. The excess diastolic force in TTNtv EHTs was abolished by 3 μM mavacamten, while second harmonic generation (SHG) microscopy of EHTs revealed greater collagen density in TTNtv EHTs.

Conclusions: Titin insufficiency in EHTs leads to blunted length-dependent activation and reduced contractile force, both of which can be restored by ABE. *TTN* truncation also leads to excessive myosin crossbridge activity during diastole and enhanced collagen synthesis.

Poster #16

Impact of Lmod2 on Thin Filament Structure

Gerrie P. Farman[1], Tania M. Larrinaga[1,2], Carol C. Gregorio[2], Christopher T. Pappas[1]

[1] Molecular Cardiovascular Research Program, University of Arizona, Tucson, Arizona 85719

[2] Cardiovascular Research Institute, Icahn School of Medicine, New York, New York 10029

The development of tension within striated muscle depends on the coordinated interaction of thick and thin filaments. Many factors can affect this interaction such as the amount of calcium, which is required to "activate" the thin filament, the amount of overlap between the thick and thin filaments, and many post-translational modifications to the myofilament proteins. One protein that may play a role in this interaction is Leiomodin (Lmod), which is an actin binding protein found at the pointed end of thin filaments; Lmod has been shown to elongate the filaments. Recently, we demonstrated that Lmod2, an isoform found primarily in cardiac and slow skeletal muscles, directly impacts the acto-myosin interaction independent of the length of the thin filament. This observation led us to predict that Lmod2 alters activity of the myofilament lattice to regulate muscle contractility. Wild-type and constitutive Lmod2 knock-out (KO) mice were injected with AAV9-GFP Lmod2. This approach rescues the cardiac phenotype (typified by contractile dysfunction and dilation) of the Lmod2 KO mice, but leaves their skeletal muscles without any Lmod2. Using the Advanced Photon Source at Argonne National Labs we exposed the chemically skinned soleus muscles to various amounts of external calcium and collected 1 second exposures for analysis. We observed that the thick filament was unaffected by the presence or absence of Lmod2, while the thin filament demonstrated a dependence on the presence of Lmod2. When activated, the 1st troponin meridional reflection moved in WT fibers, while it remained stationary in fibers from KO animals. Furthermore, addition of recombinant Lmod2 to the fibers did not restore the change in spacing with addition of calcium. Contrary to the spacing of the meridional patterns, the intensities, a measure of ordering, demonstrated a greater decrease with activation in the KO fibers than in the WT fibers; this was reversed when recombinant Lmod2 was added. We conclude that the presence of Lmod2 is important for ordering the thin filament and regulating the movement of the Tpm/Tn complex. In addition, the spacing of the Tpm/Tn complex requires Lmod2 present during the maturation of the thin filament.

Poster #17

Investigation of DCM-mutation (*ACTC1*-E361G) with Blunted Response to β-Adrenergic Stimulation in Mice

Ronnie Chastain[1], Andrew Reilly[1], Olivia Rose Edwards[1], Kealyn Bowie[1], Raissa Arraval[1], Amelia Koopman[1], Riley Dehlinger[1], Gannon Walcott[1], Lilly Wiristiuk[1], Steven Marston[2], and Jose R. Pinto[1]

[1]Florida State University College of Medicine, Biomedical Sciences Department

[2]Imperial College of London, National Heart and Lug Institute Division

Dilated cardiomyopathy (DCM) refers to the maladaptation of the cardiac musculature, which is characterized by left ventricular enlargement and systolic dysfunction. Approximately half of non-ischemic heart failure cases in the U.S. are DCM and of those, 30-35% are due to underlying genetic causes. Many different mutations of the *ACTC1* gene are known to cause DCM; however, the mutation E361G also reduces the responsiveness of the cardiac thin filament (cTF) to β-adrenergic stimulation. Downstream signaling subsequent β-adrenergic stimulation leads to the phosphorylation of serines 23/24 on the inhibitory unit of the troponin complex, cTnI. This post-translational modification allows for an increased rate of relaxation, due to Ca^{2+} desensitization of cTnC, that helps the heart compensate for the increased blood volume needed to perfuse the periphery. In this present study, we will leverage a transgenic murine model containing the *ACTC1*-E361G mutation to characterize this model *in vivo* and how it responds to β-adrenergic stimulation. Mice, 3- and 6-months of age, were anesthetized before heart structure/ function was assessed via echocardiography. After baseline metrics were collected, subsequent IP injections of dobutamine (0.25, 0.5, 0.75 mg/kg) were administered to chemically mimic β-adrenergic stimulation. Although baseline measurements, prior to injections, were not different between non-transgenic (Ntg) and Transgenic (Tg) specimen; however, repeated injections revealed an inability to respond to the increased cardiac demand initiated via β-adrenergic stimulation. This model would be useful in determining how those with this mutation, and others that inhibit the appropriate response to acute stress, develop cardiac maladaptation that generate DCM.

Rules of the Rod: *MYH7* Variant Effect Across the β-MHC Coiled-Coil Domain

Clayton E. Friedman 1,2,3, Shawn Fayer 4, Sriram Pendyala 4, Wei-Ming Chien 1,2,3,5, Charles E. Murry 1,2,3,6,7, Lea M. Starita 4,8, Douglas M. Fowler 4,7,8, Kai-Chun Yang 1,2,3,5

1 - Institute for Stem Cell and Regenerative Medicine, University of Washington, School of Medicine, Seattle, WA 98109, USA.
2 - Center for Cardiovascular Biology, University of Washington, Seattle, WA 98109, USA.
3 - Department of Medicine/Cardiology, University of Washington, Seattle, WA 98109, USA.
4 - Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA.
5 - Cardiology/Hospital Specialty Medicine, VA Puget Sound HCS, Seattle, WA 98108, USA.
6 - Department of Pathology, University of Washington, Seattle, WA 98109, USA.
7 - Department of Bioengineering, University of Washington, Seattle, WA 98195, USA.
8 - Brotman Baty Institute for Precision Medicine, Seattle, WA 98195, USA.

Background: *MYH7* encodes β-MHC, a thick filament protein in striated myocytes. β-MHC contains a head domain and a coiled-coil rod domain with a repetitive heptad motif. Pathogenic *MYH7* missense variants are clinically actionable because they assist with the diagnosis and cascade screening of individuals at risk of hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, and skeletal myopathies. However, most *MYH7* variants are of uncertain significance (VUS), limiting the utility of genetic testing. We established a high-throughput phenotyping pipeline and predictive model to prospectively determine *MYH7* variant effects at scale.

Methods: We used CRISPR-Cas9 to knock a *MYH7* single nucleotide variant library spanning six consecutive heptads of the β-MHC coiled-coil domain into the endogenous *MYH7* locus of human induced pluripotent stem cells. We differentiated this pooled library to cardiomyocytes, used FACS with next-generation sequencing to measure β-MHC-meGFP abundance for each variant, and correlated β-MHC abundance to clinical HCM phenotypes. We used β-MHC abundance data to build a computational model for predicting *MYH7* variant effects across the rod and independently validated this model using UK Biobank data. We also examined the model's generalizability in coiled-coil domains of unrelated proteins.

Results: Abnormal β-MHC abundance accurately segregated known pathogenic (n=25) and benign (n=18) *MYH7* variants (sensitivity 0.83; specificity 0.81) reaching Moderate strength OddsPath and OddsBenign scores, supporting classification of 15 VUS to likely pathogenic and 13 VUS to likely benign. β-MHC abundance correlated with left ventricular wall thickness and age of HCM onset. We observed that β-MHC abundance is dependent on amino acid position within the heptad motif and leveraged these granular structure-function data to predict the effects of 17306 *MYH7* variants spanning 1044 amino acids of the β-MHC coiled-coil domain (sensitivity 0.59; specificity 0.89), supporting classification of >1000 *MYH7* VUS. Individuals with predicted abnormal *MYH7* variants showed a 7.5-fold increase in HCM diagnosis. This *MYH7*-based model accurately segregated pathogenic and benign variants in *MYH6*, *MYH3*, *MYH2*, *KCNQ1*, *KRT1*, and *KRT10* coiled-coil domains.

Conclusions: We show disrupted β-MHC dosage in gene-edited cardiomyocytes predicts *MYH7* variant pathogenicity and provide an atlas of *MYH7* variant effects to assist with future variant classifications, bridging genetic testing with life-saving interventions.

Poster #19

AAV-Mediated Troponin Replacement Reclassifies *TNNT2* Variants and Uncovers Myocardial Plasticity of Dilated Cardiomyopathy

Yu-Chieh Chen[1,2], Tae Kyeong Kim[2], Abhijit Takawale[2], Lisa M. Wren[2], J. Travis Hinson[1,2]

[1]Calhoun Cardiology Center, University of Connecticut Health Center, Farmington CT

[2]The Jackson Laboratory for Genomic Medicine, Farmington CT

Cardiomyopathies comprise a diverse group of myocardial disorders that can lead to heart failure, arrhythmias, and sudden cardiac death. Common subtypes such as dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy often arise from missense mutations in the *TNNT2* gene, which encodes cardiac troponin T2, a critical regulator of sarcomere function. Despite its clinical importance, the cellular responses to altered troponin mechanics and the extent to which maladaptive remodeling is reversible remains incompletely defined.

To address this, we developed an integrated AAV-based platform for *in vivo* functional interrogation of *TNNT2* variants and systematic assessment of cellular plasticity in a genetic model of DCM driven by R141W-*Tnnt2* mutation. Myotropic AAV vectors were engineered to replace endogenous *Tnnt2* with *TNNT2* variants, enabling variant-specific interrogation of contractile, structural, and molecular remodeling. Our result demonstrated efficient replacement of wild type *TNNT2 in vivo* and reclassified two *TNNT2* variants previously designated as of unknown significance. This gene delivery approach improved systolic performance and mitigated pathological remodeling in DCM mice, but only partially reverse DCM signatures, and incompleteness cannot be fully explained by gene replacement efficiency. These findings suggest that more potent augmentation of cardiomyocyte contractility may be required to fully overcome the entrenched disease state. Together, this work establishes a versatile *in vivo* framework for resolving *TNNT2* variant pathogenicity and reveals intrinsic limits to myocardial plasticity, highlighting the need for therapeutic strategies that both repair causal mutations and enhance contractile capacity to overcome persistent features of genetic DCM.

Evolutionary and structural analysis reveals presence of novel domain elements in titin

Jochen Gohlke and Henk Granzier

Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA.

Titin is a sarcomeric protein spanning from the Z-disc to the M-line and acts as a molecular spring, helping center the thick filament and restore sarcomere length after stretch. Its backbone is a linear array of immunoglobulin-like (Ig) and fibronectin type III (Fn3) domains, which provide modular blocks that can sequentially unfold under force to absorb stretch.

Throughout the protein, there are stretches of unique sequences of different lengths which were mostly thought to be unstructured until recently, when a novel structural domain was discovered in titin's N2B segment. We used a combination of sequence comparisons and structural predictions using Alphafold 3 on over 150 known titin orthologs across vertebrates. In addition to confirming the presence of the N2B structural element, we discovered high-confidence predictions of alpha-helical elements in titin's Z-disk and M-band regions, as well as a novel domain in titin's novex-3 exon.

The novel element in titin's Z-disk is predicted to be positioned adjacent to titin's Z4 domain. Similarly, the other alpha-helical structure in titin's M-band is predicted to be positioned very close to domain M6. Both alpha-helices might be involved in protein-protein binding or mechanical buffering and strain absorption and thereby influence Ig domain properties. The novel domain in titin's novex-3 exon is located between Ig domains 4 and 5 and could also be involved in protein-protein interactions.

For titin's A-band region, we found that the Ig- and Fn3-domain pattern, starting with the IA junction and ending with the P-zone, was identical across over 600 vertebrate titin orthologs, indicating that the A-band domain pattern has been conserved for >500 million years despite only ~45% amino acid identity. This indicates that A-band patterning, which forms a molecular template that fixes thick filament length, spacing of myosin heads, and positioning of myosin-binding protein C (MyBP-C), is under extremely strong functional constraint. Interestingly, we found the number of titin's M-domains to be variable across vertebrates, with 10 M-domains in mammals but only 9 in reptiles, birds and most amphibians. Fish isoforms include only seven 7 M-domains in isoform TTN.1 and 9 M-domains in TTN.2.

Mutation-related efficacy of novel therapeutic approaches in hypertrophic cardiomyopathy

***Dente M.*[1], Santini L.[2], Pioner J.M.[3], Palandri C.[2], Scellini B.[1], Piroddi N.[1], Tardiff J.[4], Cerbai E.[2], Poggesi C.[1], Tesi C.[1], Ferrantini C.[1], Coppini R.[2], De Tombe P.P[5,1].**

[1]Dept. of Experimental and Clinical Medicine, University of Florence, Florence, Italy.
[2]Dept. of NeuroFarBa/ University of Florence- Italy.
[3]Department of Biology, University of Florence, Florence, Italy.
[4]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, Arizona.
[5]Phymedexp, Université de Montpellier, Inserm, CNRS, Montpellier, France.

BACKGROUND: The myosin inhibitor Mavacamten (MAVA) and the late Na^+ current blocker Ranolazine (RANO) represent two novel targeted therapeutic options for hypertrophic cardiomyopathy (HCM). Although both drugs have shown beneficial effects on diastolic dysfunction and arrhythmias, it remains unclear whether their efficacy is influenced by the patient's genetic background.

AIM: To evaluate whether genetic background modulates drug response by analysing the impact of MAVA and RANO in two mouse models carrying different mutations in the TNNT2 gene: the R92Q mutation, which enhances diastolic cross-bridge activity by increasing diastolic intracellular Ca^{2+} levels, and the E163R mutation that is associated with increased tension cost and Ca^{2+}-independent baseline cross-bridge activity.

METHODS: The two drugs (0.5-1 μM, MAVA, and 10μM, RANO) were tested on skinned multicellular preparations to assess force and ATPase activity and on intact cardiomyoctes to assess intracellular calcium fluxes.

RESULTS; In both genetic models, MAVA significantly reduced maximal Ca^{2+}-activated tension and resting tension as well as maximal and resting ATPase activity, without detectable effects on Ca^{2+} handling. The reduction in ATPase activity was largest in the E163R mutant. In contrast, RANO accelerated Ca^{2+} transient kinetics only in R92Q myocardium, consistent with the presence of the increased late Na^+ current and prolonged Ca^{2+} transients observed only in this mutation.

CONCLUSIONS: MAVA normalised tension cost in the E163 mutant, while RANA improved diastolic function only in R92Q. These data highlight the need for mechanistic cellular insights to aid development of "patient personalised" treatment strategies.

Poster #22

Methylglyoxal induces sarcomere dysfunction and impairs relaxation in the heart

Henry M. Gong[1], Stephanie Deditz[1], Christine Delligatti[2], Michaela Door[1], Sarah Flury[3], Micah Yoder[1], Kathleen Woulfe[4], Jonathan A. Kirk[1]

1. Biological Science Division, University of Chicago Medicine, Chicago, IL
2. Department of Medicine, The John Hopkins University, Baltimore, MD
3. Department of Cell & Molecular Physiology, Loyola University Chicago, Maywood, IL
4. Department of Medicine, University of Colorado Anshutz, Aurora, CO

Advanced glycation end-products (AGEs) accumulate in aging, diabetes mellitus, and cardiomyopathy. Glycation is an irreversible, non-enzymatic post-translational modification on lysine and arginine residues. The reactive dicarbonyl, glyoxal (GO) and methylglyoxal (MGO), are the major glycating agents and are elevated in disease states. Here, we investigate the impact of MGO on cardiac function, protein expression, and sarcomere structure using intact rat myocardial slices, mass spectrometry (MS), and x-ray diffraction.

Building on our previous work demonstrating sarcomere dysfunction after exposure to 100 μM of MGO in both human and mice left ventricle (LV) skinned cardiomyocytes, we exposed intact rat LV myocardial slices to 1 μM or 100 μM MGO overnight at 4°C, then each slice was stretched to sarcomere length of 2.1 μL and stimulated in room temperature. Slices treated with 1 μM MGO were indistinguishable from controls. In contrast, 100 μM MGO significantly slowed relaxation, and work-loop analysis revealed a substantial decrease in the end-diastolic force-length relationship (EDFLR), with no change in active tension and end-systolic force-length relationship (ESFLR). Mass spectrometry showed proteins involved in metabolism and Ca^{2+} reuptake were differentially expressed following 100 μM treatment. Both 1 μM and 100 μM MGO increased glycation of sarcomere proteins such as troponin-T, actin, myosin, titin, and myosin light chain. X-ray diffraction of resting slices showed a subtle increase in intensity ratio without changes in lattice spacing, suggesting a shift in myosin heads toward the thin filament.

To isolate the sarcomere effect of MGO, we performed force–Ca^{2+} experiment in skinned rat cardiomyocytes exposed to 100 μM MGO for 20 minutes. This treatment significantly reduced maximal tension without altering calcium sensitivity or cooperativity. To determine whether slowed relaxation was due to sarcomere mechanics, single mouse myofibrils were exposed to 100 μM MGO, which significantly slowed the linear decay during relaxation. Finally, x-ray diffraction in skinned pig myocardium at rest showed a significant increase in intensity ratio without changes in lattice spacing following 100 μM treatment consistent with our interpretation of intact rat myocardial slices.

Together, these findings indicate that 100 μM MGO induces sarcomere dysfunction and impairs relaxation through disrupting the thick-filament resting structure and its interaction with the thin filament during contraction, with possible additional contributions from prolonged calcium re-uptake.

Poster #23

Impaired Myofibril Relaxation in a Porcine Model of cTnI-Associated Hypertrophic Cardiomyopathy is Rescued with AAV Gene Therapy

Axel Fenwick[1], Darla Tharp[2], Bhanu Telugu[2,3], Elizabeth Silver[4], Betul Gunes[4], Julius Bogomolovas[4], Eryn Wagoner[2], Brittany Mezzancella[2], Ashley Clark[2], Sean Simpson[2,3], Ki Eun Park[2,3], Jerel Waters[2,3], Claire Geddes[5], Kevin King[4], Ju Chen[4], Eric Adler[4,5], Anthony Cammarato[1]

1 Johns Hopkins University, Baltimore, United States, 2 University of Missouri, Columbia, United States, 3 RenOVAte Biosciences, Columbia, United States, 4 University of California San Diego, San Diego, United States, 5 Lexeo Therapeutics, New York, United States

TNNI3 encodes cardiac troponin I (cTnI), a subunit of the thin-filament troponin complex that inhibits contraction by stabilizing tropomyosin in a state that blocks actomyosin cross-bridge interaction at rest. cTnI loss-of-function mutations frequently cause hypertrophic cardiomyopathy (HCM) and plausibly trigger the hypercontractile phenotype and diastolic dysfunction that characterize the disease. Nevertheless, the molecular mechanisms underlying HCM-associated relaxation deficits remain incompletely understood. Prior studies have shown that HCM mutations often impact relaxation of isolated myofibrils, the fundamental contractile organelles of muscle. Myofibrillar relaxation is distinctly biphasic, consisting of an initial slow, linear phase followed by a fast, exponential phase. The slow phase reflects the time required for the number of cycling cross-bridges to drop below the threshold for cooperative thin-filament activation, which is routinely prolonged in HCM, while the fast phase corresponds to the detachment of remaining cross-bridges and recoil of elastic elements. Here, we generated a homozygous cTnI A158V HCM porcine model displaying early-onset cardiac hypertrophy, atrial dilation, preserved ejection fraction, and complete lethality within two months. Administration of an AAV vector expressing full-length cTnI significantly attenuated hypertrophy and atrial dilation and prevented early mortality. However, organ-level improvements do not ensure rectification of underlying molecular deficiencies. Therefore, we used a custom-built biomechanics platform to measure the force and kinetics of individual cardiac myofibril activation and relaxation. Consistent with prior studies, the duration of the slow relaxation phase was ~40% prolonged in cTnI A158V vs. wildtype myofibrils, supporting impaired thin-filament inactivation as a molecular basis for diastolic dysfunction. This defect was corrected in AAV-treated animal myofibrils. For comparison, we also assessed the cardiac myosin inhibitor mavacamten (MAVA), which shifts myosin heads toward detached states and accelerates both relaxation phases in non-failing human myofibrils. 1 μM MAVA significantly reduced tension across all groups but failed to improve the impaired slow phase in cTnI A158V myofibrils. These findings demonstrate that cTnI A158V impairs the slow, linear phase of myofibrillar relaxation in our porcine HCM model, consistent with defective thin-filament inactivation, and that AAV gene therapy rectifies this deficit, while MAVA fails to do so and may additionally prompt a dangerous reduction in force.

Energy Consumption After Active Stretch in Single Skinned Muscle Fibres: Titin or No Titin?

Torri Heiser*, Venus Joumaa, and Walter Herzog
Human Performance Lab, Faculty of Kinesiology, University of Calgary, Calgary, Canada

Introduction

The isometric force produced at steady-state following active stretching is greater than a purely isometric contraction at the same final muscle length and level of activation. This muscle property has been widely observed and is known as residual force enhancement (rFE) (e.g., [1]). Despite decades of research, the exact molecular mechanisms underlying rFE are not fully understood. However, there is increasing evidence that titin may contribute to rFE [1]. If so, the increased force in rFE would occur with little or no increase in energy (ATP) consumption. There is little systematic research in which ATP consumption has been measured in the rFE state [2].
The purpose of this study was to determine the ATP cost in skeletal muscle fibres in the rFE state compared to the corresponding isometric reference state. We hypothesized that if titin contributes to rFE, the ATP cost per unit of force should be less in the enhanced compared to the corresponding reference state.

Methods

Single skinned rabbit psoas fibres (n=12) were used to measure the rate of ATP consumption during steady-state isometric contractions at an average sarcomere length of 3.2μm and during steady-state isometric contractions following active stretch from an average sarcomere length of 2.4μm to 3.2μm. Force and ATP use were measured synchronously using a single fibre testing apparatus (1415A and 804A, Aurora Scientific) as described by de Tombe and Stienen [3].

Results

Our results show an average rFE of 11±0.6% and a 20±7% decrease in ATP consumption per unit of force following active stretch compared to the purely isometric reference contractions.

Discussion

The reduced ATP consumption per unit of force in rFE compared to the corresponding isometric contraction supports the idea that the extra force in rFE originates from a passive element and not from an increase in cross-bridge activity. Titin-calcium and titin-actin interactions have been suggested to occur upon muscle activation and stretch, resulting in an increase in titin stiffness, and thus rFE without a corresponding increase in ATP consumption.

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Poster #25

Variants in skeletal muscle actin as potent disruptors of cardiac contractility

Ankit Garg*[1], Fengying Li[1], Silvia Jansen[2], Lina Greenberg[2], Gretchen A. Meyer[3], Rui Zhang[4], Kory J. Lavine[5], Michael J. Greenberg[4]

*Corresponding author, [1]Department of Medicine at Johns Hopkins Medicine & [2]Department of Cell Biology and Physiology, [3]Program in Physical Therapy, [4]Department of Biochemistry and Biophysics, [5]Center for Cardiovascular Research at Washington University School of Medicine

Skeletal muscle actin (ACTA1) is robustly expressed in skeletal muscle but present in trace amounts in the heart. While ACTA1 variants are established causes of myopathies, there are rare reports of ACTA1 variants associated with cardiomyopathy which have controversial pathogenicity. We previously reported the association of the R256H ACTA1 variant with dilated cardiomyopathy (DCM), but it was unknown whether this variant is pathogenic. Here, we establish a causative link between R256H ACTA1 and DCM through multiscale analysis utilizing animal and cellular models alongside biochemical and structural analysis of purified proteins.

To establish pathogenicity, we created an *Acta1*$^{R256H/+}$ knock-in mouse. These mice had no baseline cardiac defects but exhibited skeletal muscle weakness consistent with the tissue expression pattern of Acta1. It is well known that Acta1 expression increases following cardiac injury and stress. We performed transaortic constriction resulting in Acta1 protein increasing from < 1% of total sarcomere actin to approximately 5%. Intriguingly, this was sufficient to cause *Acta1*$^{R256H/+}$ mice to develop significant cardiac hypocontractility with a corresponding increase in fibrosis. To corroborate pathogenicity in a human model, we recapitulated hypocontractility in *ACTA1*$^{R256H/+}$ human iPSC-derived cardiomyocytes.

To dissect how R256H ACTA1 causes potent contractile defects, we purified recombinant R256H ACTA1. Molecular dynamics simulation suggested that R256H ACTA1 had decreased nucleotide binding. Accordingly, purified R256H ACTA1 demonstrated decreased thermal stability, nucleotide exchange, and polymerization kinetics. Next, we directly evaluated the effect of R256H ACTA1 on biochemical contractility. In the absence of calcium regulation through the troponin-tropomyosin (Tn-Tpm) complex, R256H ACTA1 filaments had unchanged motility with cardiac myosin. Upon addition of Tn-Tpm, R256H ACTA1 filaments exhibited arrested motility and small amounts of R256H ACTA1 in WT ACTA1 filaments was sufficient to disrupt motility. To understand the structural changes responsible for this phenomenon, we resolved the structure of the R256H ACTA1 filament by cryo-EM. We found a disrupted interaction between ACTA1 K240 and tropomyosin E117, a residue important for maintaining Tpm in a state open to actin-myosin interactions. We believe this disrupted interaction contributes to decreased biochemical contractility in R256H ACTA1-containing filaments.

Collectively, we show that an *ACTA1* variant, R256H, is capable of causing DCM through potent defects in multiple actin biochemical functions. This effect is likely related to structural changes altering actin-Tpm interactions. We believe other ACTA1 variants associated with cardiomyopathy are likely potent disruptors of sarcomere contractility.

New Capabilities of the MuscleX Data Reduction Package for Muscle Diffraction

Xinyu Lan[1], Irina Klein[2], Gady Agam[2], and Thomas C. Irving[1]

[1]BioCAT, Department of Biology, and [2]Department of Computer Science, Illinois Institute of Technology, Chicago, IL 60616

For many years, analysis of X-ray fiber diffraction patterns from muscle was a rate limiting step in the preparation of experimental results for publication. Most analysis procedures made extensive use of FIT2D supplemented by locally developed or commercial software tools which were not specifically designed for the task. The open source "MuscleX" data reduction suite (Jiratrakanvong et al., *J. Synchrotron Rad.* (2024). 31, 1401–1408). was written to address this bottleneck. Installers permit easy deployment under MS Windows and Linux while conda and pip versions can be installed in python virtual environments in other operating systems including MacOS. The "Equator' routine can analyze the equatorial patterns from a complete experiment in a few hours without operator intervention. The 'Quadrant-Fold" package averages the four quadrants of a diffraction pattern to improve signal to noise and remove gaps between modules in common pixel array detectors. "Projection Traces" can be used to analyze the intensity and spacings of prominent reflections on the meridian and layer lines. "Add Intensities" be used to bin frames together as well as combine data from different time resolved experiments. The entire package has been completely rewritten (v1.28) to enhance performance with multiprocessing, with many enhancements to improve the accuracy of measurements. The overall code base makes extensive use of reusable widgets that are used across modules to reduce bugs, improve the user experience and reliability and make the code easier to maintain. Protocols in projections traces can be saved and restored to improve efficiency and reproducibility. An important new development is the ability to analyze clusters of peaks including interference splitting of the myosin meridionals and the overlapping peaks in the M1 and M2 clusters including the so-called C-protein reflections. Another major development is a greatly improved global 2D background subtraction protocol that can determine the best algorithm with optimized parameters to allow easier analysis of layer line data. (Supported by NIH R01GM144555)

Altering the ratio of reduced to oxidized glutathione affects contractile kinetics in cardiomyocytes

Greenman, A.C.[1], Farrell, E.T.[1], De Lange, W.J.[1], Langlais, P.R.[2,3], Harris, S.P.[4], Ralphe, J.C.[1]

[1]Department of Pediatrics, University of Wisconsin-Madison, Madison, WI

[2]Department of Medicine, Endocrinology Division, University of Arizona, Tucson, AZ

[3]Quantitative Proteomics Laboratory, University of Arizona, Tucson, AZ

[4]Department of Physiology, University of Arizona, Tucson, AZ

S-glutathionylation is a reversible post-translational modification that is increased when the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is decreased, seen in cardiovascular disease. The S-glutathionylation of cardiac myosin binding protein-C (cMyBP-C) may prolong relaxation of the heart. Thus, the reversal of S-glutathionylation may serve as a new therapeutic target in treating diastolic dysfunction. Here, we aimed to determine the impact of cMyBP-C S-glutathionylation on cardiac function using both a permeabilized fiber and an intact cardiomyocyte approach.

S-glutathionylation of cMyBP-C was confirmed by western blot and tandem mass spectrometry. Cardiac trabeculae from wild-type mice were membrane-permeabilized, attached to a motor and force-transducer to measure force before and after incubating with 5mM of GSSG. Engineered heart tissue (EHT) constructs were generated using cardiomyocytes derived from control, cMyBP-C ablated, and a hypertrophic cardiomyopathy mutant, human induced pluripotent stem cell (hiPSC) lines. EHT twitch force was measured during 1 Hz pacing using the MantArray system (Curi Bio). Since GSSG is impermeable, we treated intact EHT with the cell permeable GSH (1mM) to test the effect of increasing the GSH/GSSG ratio (favoring the de-glutathionylation of protein targets). Permeabilized fibers were compared using a paired t-test and EHT groups were compared using a two-way ANOVA, significance set to a p-value < 0.05.

Incubating permeabilized cardiomyocytes in GSSG increased the S-glutathionylation of cMyBP-C compared to vehicle and slowed the rate of force redevelopment ($n = 4$, $p = 0.02$). Treating EHT with GSH reduced peak twitch force ($n = 9$-10/treatment, $p = 0.01$) and slowed the time to 50% of relaxation ($p = 0.03$) regardless of genotype. Unexpectedly, both GSSG and GSH treatment slowed cardiomyocyte kinetics in a permeabilized and intact system, respectively. These differential functional findings suggest that an altered GSH/GSSG ratio may modify proteins other than cMyBP-C and override the functional effects of cMyBP-C S-glutathionylation. Alternatively, increasing the bioavailability of GSH might not reverse cMyBP-C S-glutathionylation. Future studies are underway to confirm the level of S-glutathionylation of other cardiomyocyte protein targets and to directly test the GSH/GSSG ratio before and after GSH treatment.

Poster #28

Structural changes in the thick and thin filaments during slow stretch of mammalian skeletal muscle

Cameron Hill[1], Michaeljohn Kalakoutis[1], Alice Arcidiacono[1], Yanhong Wang[1], Atsuki Fukutani[1], Theyencheri Narayanan[2], Elisabetta Brunello[1], Luca Fusi[1.3], Malcolm Irving[1]

[1]Randall Centre for Cell & Molecular Biophysics and British Heart Foundation Centre of Research Excellence, Guy's Campus, King's College London, London, SE1 1UL

[2]European Synchrotron Radiation Facility, 38043 Grenoble, France

[3]Centre for Human & Applied Physiological Sciences, Shepherd's House, Guy's Campus, King's College London, London, SE1 1UL.

Skeletal muscle can produce large braking forces while sarcomeres are extended very slowly. The braking function of muscles is important for everyday actions like walking downstairs, but its molecular structural basis remains largely uncharacterised. We applied a ramp stretch of 2.5% muscle length in 65 ms to mouse EDL muscles after peak fixed force had been established during tetanic contraction at 27°C and determined the accompanying structural changes in thick and thin filaments by time-resolved X-ray diffraction. Force increased by 40% during the stretch, and sarcomere length increased by 40 nm/half-sarcomere with little increase in sarcomere inhomogeneity. Relaxation at the end of stimulation was slowed, and exponential relaxation started about 20ms later than in a fixed-end tetanus. The intensity of the 1,1 equatorial X-ray reflection decreased by about 30% during the stretch, with no change in that of the 1,0 reflection, suggesting that thin filaments were displaced from their trigonal positions in the hexagonal filament lattice. There was no change in the intensity of the myosin-based layer lines or in the spacing of any of the myosin-based axial reflections during the stretch, indicating that the regulatory state of the thick filament was not altered by the stretch. The intensity of the second actin-based line, signalling the movement of tropomyosin associated with activation of the thin filament, increased slightly. The intensity of the first actin-based layer line, signalling the fraction of myosin motors bound to thin filaments, decreased slightly during the stretch, in apparent contradiction with the increase suggested by previous stiffness measurements. The intensity of the M3 reflection associated with the axial repeat of the myosin motors decreased during the stretch, as did the fractional intensity of its low-angle interference sub-peak, indicating a movement of the centre of mass of actin-attached motors by about 1.5 nm towards the M band, i.e. towards the start of the powerstroke. These results constrain structural and kinetic descriptions of the action of the myosin motors that underpin the braking action of muscle during active stretch.

Poster #29

Mant-ATP-Informed Patient-Specific Cardiac Mechanics in Hypertrophic Cardiomyopathy

Julia Seungyeon Han[1], Filip Ježek[2], Javiera Jilberto[1], Anna Damlin[3], David Marlevi[3], Daniel A. Beard[2], David A. Nordsletten[1,4], Alison S. Vander Roest[1]

[1] Department of Biomedical Engineering, University of Michigan, MI, USA
[2] Department of Molecular and Integrative Physiology, University of Michigan, MI, USA
[3] Department of Medical Sciences, Karolinska Institutet, Stockholm, Sweden
[4] Department of Cardiac Surgery, University of Michigan, MI, USA

Hypertrophic cardiomyopathy (HCM) is characterized by sarcomeric dysfunction, energetic inefficiency, fibrosis, and patient-specific ventricular remodeling, yet the relationship between molecular myosin-state abnormalities and organ-scale ventricular mechanics remains poorly defined. We propose a modular multiscale framework that connects mant-ATP-based kinetic modeling of myosin state behavior with patient-specific full-cycle cardiac mechanics simulations in HCM.

At the molecular scale, a mechanistic mant-ATP turnover model is used to infer reduced descriptors of myosin behavior, including force-available myosin fraction, altered ATP turnover, and changes consistent with disrupted super-relaxed/disordered-relaxed (SRX/DRX) state regulation. Rather than directly embedding the molecular state model into the organ-scale simulation, these outputs are translated into reduced modifiers of active myocardial behavior, enabling tractable cross-scale coupling while preserving mechanistic interpretability.

At the organ scale, patient-specific HCM ventricular geometry, fibrosis mapping, activation delay, passive parameter identification, and pressure-volume constraints are incorporated into a full-cycle mechanical simulation pipeline. Molecularly informed perturbations of the active contraction waveform are then introduced through activation scaling, allowing comparison of baseline, HCM-like, and energetically stressed molecular conditions within the same geometry. This framework is designed to test how altered myosin-state kinetics may influence ventricular mechanics in a geometry-dependent manner.

Primary readouts include pressure-volume behavior, stroke volume, ejection fraction, and regional stress and strain distributions, with particular emphasis on differences between fibrotic and non-fibrotic myocardium. By preserving patient-specific anatomy and hemodynamic constraints while introducing molecularly informed perturbations of active contraction, this approach provides a practical strategy for probing how sarcomeric energetic abnormalities may manifest as organ-scale dysfunction in HCM.

This study establishes a feasible path toward integrating molecular energetics and organ-level mechanics in HCM and provides a foundation for future investigations of remodeling, energetic burden, and treatment response.

Novel Chemical Protein Foot-Printing Mass Spectrometry Methodology for Identification of Thin Filament Structural Differences in Ca^{2+} Free and Activated States

Johannes V Janssens[1], Casimir Bamberger [2#], Chris I Murray[1#], Deepika Rai[1], Lea M D Delbridge[3], Sarah J Parker[1], Simion Kreimer[1], John R Yates[2,] Jennifer E Van Eyk[1]

1. Advanced Clinical Biosystems Research Institute, Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA.
2. Department of Molecular Medicine, The Scripps Research Institute, La Jolla, California, USA.
3. Department of Anatomy and Physiology, The University of Melbourne, Melbourne, Victoria, Australia.
 # Equal contribution

INTRODUCTION

Myofilaments contain intrinsically disordered regions that undergo dynamic conformational changes throughout the contractile cycle. Mutations and modifications within these regions are linked with cardiac disease. Structural information regarding the response of these regions to physiologic and pathologic stimuli remains sparse. The study's aim is to detect differences in lysine solvent accessibility across the myofilament proteome in relaxed and Ca^{2+}-activated states using a chemical protein foot-printing methodology named Covalent Protein Painting (CPP), to gain structural insights into myofilament dynamics.

METHOD

Cardiac myofibrils were mechanically liberated from 6-week-old mouse ventricle. Intact myofibrils were exposed to a buffer +/-5mM Ca^{2+} followed by the CPP protocol. Myofibrils were dimethyl labeled by adding 60mM formaldehyde and 60mM sodium cyanoborodeuteride for 15min followed by addition of 300mM ammonium acetate to quench the reaction. Following denaturation and digestion (Chymotrypsin or ArgC, 1:25), peptides were labeled with a 'heavy' dimethyl group, and data acquired on an Orbitrap Lumos (ThermoFisher) in DDA mode and quantified with ProteinClusterQuant. Ratios of light and heavy peptides (within the same sample) were computed to calculate percent solvent accessibility for each lysine site. The percentage value describes how often a particular lysine site was accessible to the labelling solvents.

RESULTS

23% (102/444) of lysine sites in myosin heavy and light-chains, actin, troponin complex, MyBP-C were labeled and quantified. Titin contributed the most labeled sites overall (377/3038). Three lysine residues within the Ca^{2+} responsive troponin complex (Troponin-I K194, Troponin-T K110, K230) exhibited >10% higher accessibility upon Ca^{2+}-addition. Notably, these sites of differential accessibility occur in 1) the Troponin-I C-terminal which is involved in modulating tropomyosin positioning in the presence of Ca^{2+} and 2) the Troponin-T N-terminal which directly binds to tropomyosin and suppresses myosin ATPase activity in low Ca^{2+} conditions also through tropomyosin positioning.

CONCLUSIONS

Preliminary findings indicate that differences in the lysine site accessibility of Ca2+-activated myofilaments can be resolved with CPP for both structured and disordered domains. Future technical studies will investigate the utility of non-specific proteases in increasing the lysine coverage of the CPP approach. CPP is a promising, novel and high-throughput approach for investigating myofilament structure in health and disease.

Small Molecule Compounds that Restore Cardiac Thin Filament Calcium Exchange Improve Myocellular Function in a Murine Model of Hypertrophic Cardiomyopathy

Garrett T. Hauck[1], Romi Castillo[2], Rhyaan Mohran[2], Melissa L. Lynn[2], Jil C. Tardiff[1,2]

[1]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ

[2]Department of Biomedical Engineering, University of Arizona, Tucson, AZ

Hypertrophic cardiomyopathy (HCM) is a disease of the cardiac sarcomere, often resulting from point mutations in thick and thin filament (TF) proteins. The development of mavacamten and aficamten has demonstrated that therapeutics targeting the sarcomere are viable, however both compounds are only approved for obstructive HCM, leaving ~1/3 of HCM patients without targeted therapies. Our lab has shown that proper regulation of the rate of Ca^{2+} dissociation from the TF is crucial for sarcomeric function and modulation, and that this rate is often altered by TF mutations. We hypothesize that Ca^{2+} dissociation rate is a targetable property of the TF and thus designed a structure-based high-throughput screen to identify compounds that bind the TF and modulate the Ca^{2+} dissociation rate. Here, we aim to apply this screen to the HCM associated R92W mutation in cardiac troponin T (cTnT-R92W), which accelerates TF Ca^{2+} dissociation. First, stopped-flow Ca^{2+} dissociation kinetics confirmed that molecules which slow Ca^{2+} dissociation in wild type TFs also significantly slow Ca^{2+} dissociation in cTnT-R92W TFs. Next, we used isothermal titration calorimetry to confirm that a candidate compound, P2O22, binds to both WT and cTnT-R92W troponin complexes with nanomolar affinity. We then treated primary cardiomyocytes isolated from cTnT-R92W transgenic mice and their Nontransgenic (NT) littermates with P2O22 to evaluate its effects on contractility and calcium handling. Preliminary and previous data indicate that cTnT-R92W cells display slower contraction and relaxation rates compared to NT cells, and acute P2O22 treatment significantly accelerates these rates. Acute P2O22 treatment also accelerates relaxation in NT cells, and does not change resting sarcomere length or percent shortening in either group. Previous data and preliminary data here has shown that cTnT-R92W cells exhibit elevated diastolic Ca^{2+}, and acute P2O22 treatment reduces diastolic Ca^{2+} levels in these cells and in NT cells, with minimal changes to Ca^{2+} transients otherwise. Taken together, these data show that acute modulation of Ca^{2+} dissociation can improve contractility and diastolic Ca^{2+} levels in HCM cells. Future work will include further testing of other small molecule candidates with isolated cardiomyocytes from cTnT-R92W and other transgenic mice, and *in vivo* treatment of cTnT-R92W mice.

Title: Titin's N2A Region Impacts Cardiomyocyte Size and Cardiac Remodeling

Elaine Kang[1], Robbert van der Pijl PhD[1], Henk Granzier PhD[1]
[1]University of Arizona, Tucson, AZ, USA

Background:
Titin is a key structural and regulatory protein in heart muscle cells (cardiomyocytes) and plays an essential role in maintaining cardiac function. Abnormal titin expression is strongly associated with heart failure, particularly dilated cardiomyopathy (DCM), where titin gene (TTN) variants may account for up to 50% of inherited cases. The heart primarily expresses two titin isoforms: N2B (stiffer) and N2BA (more compliant). DCM is often accompanied by a shift toward greater N2BA expression, but the mechanisms remain unclear. A unique segment within N2BA—called the N2A element—acts as a signaling hub and may interact with CARP1, a known regulator of cardiac growth.

Objective:
To test whether the N2A element of N2BA titin regulates heart size through interaction with CARP1.

Methods:
We used two genetically modified mouse models: 1. $Ttn^{\Delta N2A\ I80\text{-}I81}$, which lacks the N2A element of N2BA titin but leaves N2B unaffected. 2. CARP1 knockout (KO) mice. Heart tissue was analyzed by western blot for protein expression and morphometric analysis was performed on individual cardiomyocytes isolated from the left ventricle (LV). Hearts were perfusion-fixed in a relaxed state to standardize measurement conditions.

Results:
The $Ttn^{\Delta N2A\ I80\text{-}I81}$ mice showed modest cardiac atrophy (heart weight: 115.1±1.4 mg vs. 126.4±1.9 mg in wild type), primarily affecting the ventricles. Cardiomyocytes from these mice were smaller, especially shorter in length (114.5±2.3 µm vs. 131.3±5.3 µm). CARP1 protein levels were reduced in $Ttn^{\Delta N2A\ I80\text{-}I81}$ hearts, suggesting that the N2A element stabilizes CARP1 expression. However, CARP1 KO mice did not show similar decreased cardiomyocyte length, indicating that N2A regulates cell length via mechanisms beyond just CARP1.

Conclusion:
Titin's N2BA isoform—and specifically its N2A element—plays a role in controlling cardiomyocyte size, particularly length. While N2A may influence CARP1 stability, the regulation of cell size appears largely CARP1-independent. These findings shed light on how changes in titin isoform composition contribute to cardiac remodeling in cardiomyopathy and suggest novel mechanistic targets for understanding and potentially treating heart failure.

326 words, 2222 characters.

Poster #33

Age-Associated ECHS1 Loss and Sarcomeric Crotonylation Impair Myofibril Relaxation and Mitochondrial Reserve in Female Hearts

Christopher W. Hoffer[1,3], Lori A. Walker[1], Anastacia M. Garcia[1,2,3], Timothy A. McKinsey[1], Kathleen C. Woulfe[1,3]

[1] Department of Medicine, Division of Cardiology, University of Colorado, Anschutz Medical Campus, Aurora, CO

[2] Division of Cardiology, Department of Pediatrics, Children's Hospital of Colorado, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA

[3] Integrated Physiology Program, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, USA

Diastolic dysfunction increases with age and disproportionately affects women, yet the molecular mechanisms that connect age-related metabolic changes to impaired myofilament relaxation remain poorly defined. Because lysine crotonylation is a metabolically derived post-translational modification that can alter protein function, we tested whether age-associated changes in crotonyl-CoA contribute to myofibril dysfunction in female hearts.

Myofibrils isolated from non-failing female human donor hearts from women older than 60 years exhibited prolonged relaxation compared with younger adult groups. These findings were recapitulated in myofibrils isolated from the hearts of aged female mice. Preliminary proteomic analysis of myofibril-enriched fractions from female human hearts revealed sexually dimorphic reduced expression of short-chain enoyl-CoA hydratase 1 (ECHS1), a mitochondrial enzyme that catabolizes crotonyl-CoA. Immunoblot analysis demonstrated increased lysine crotonylation in aged female mouse hearts. To test whether elevated crotonylation is sufficient to impair cardiomyocyte function, adult rat ventricular myocytes were treated with sodium crotonate to increase intracellular crotonyl-CoA availability. Sodium crotonate treatment increased protein crotonylation, slowed cardiomyocyte relengthening, prolonged myofibril linear phase relaxation, and reduced maximal and spare mitochondrial respiratory capacity during palmitate-supported respiration. Together, these findings support a model in which age-associated loss of ECHS1 promotes crotonylation of sarcomeric and mitochondrial proteins, leading to impaired myofibril relaxation and reduced energetic reserve.

This work identifies crotonylation as a candidate mechanism linking mitochondrial substrate metabolism to myofilament dysfunction in aging female hearts and suggests that enzymes controlling crotonyl-CoA metabolism may represent new therapeutic targets for diastolic dysfunction.

Z-disk thickness regulation is independent of titin and nebulin Z-repeats

Justin Kolb, Xavier Aguilar, Jochen Gulke, Zaynab Hourani, Robbert van der Pijl, John Smith, Henk Granzier

Department of Cellular and Molecular Medicine, University of Arizona

The Z-disk is a protein-rich structure that plays a crucial role in transmitting both active and passive forces between sarcomeres by anchoring titin and actin thin filaments. Its thickness varies across muscle types, ranging from ~ 50 nm in fast skeletal muscles to ~100 nm in cardiac and slow skeletal muscles. Although the mechanisms regulating Z-disk thickness remain unclear, it has been hypothesized that the myofilament proteins titin and nebulin, which span the Z-disk, may act as regulators. This idea is based on the presence of variable numbers of repeat motifs in each protein: titin contains up to seven Z-repeats (Zr), with Zr1-3 and Zr7 found in all striated muscles, and Zr4-6 expressed only in cardiac and slow skeletal muscles—where Z-disks are thickest. Similarly, nebulin includes 18 Zr, with more expressed in muscles that have thicker Z-disks. To test whether titin and nebulin determine Z-disk thickness, we generated mouse models lacking all titin Zr ($Ttn^{\Delta 8\text{-}14}$), another lacking nebulin's differentially expressed Zr ($Neb^{\Delta 152\text{-}160}$), as well as a double mutant to explore co-regulation. All three models survived to adulthood and appear normal. Unexpectedly, electron microscopy revealed that Z-disk thickness in the EDL (fast), soleus (slow), and cardiac muscle of $Ttn^{\Delta 8\text{-}14}$ mice was unchanged compared to wild-type. Immunofluorescence confirmed that titin's T12 epitope, located at the Z-disk, remained properly positioned despite the titin Zr deletion. In the $Neb^{\Delta 152\text{-}160}$ model, the number of nebulin Zr in the soleus matched that of the EDL, yet Z-disk thickness remained soleus-like—again contradicting expectations. The double mutant similarly retained normal soleus Z-disk structure. Although overall sarcomere structure was maintained, $Ttn^{\Delta 8\text{-}14}$ skeletal muscle exhibited signs of myopathy, including central nuclei, increased susceptibility to eccentric contraction-induced force loss, and subsequent sarcolemmal damage as evidenced by Evans blue dye uptake. Collectively, these findings refute the hypothesis that titin and nebulin determine Z-disk thickness. Instead, Zr expression enables these proteins to span Z-disks of varying thickness and in the case of titin, contribute to membrane integrity and calcium homeostasis.

Patient-derived iPSC models of HCM reveal acute and chronic mavacamten responses in 2D iPSC-CMs and 3D engineered heart tissues

Vincent A.J. Warnaar[1,2], Floor van den Dolder[1,2], Remco Hoogervorst [1,2], Heleen Hakkeling[1,2], Diederik W.D. Kuster[1,2], Jolanda van der Velden[1,2]

1: Amsterdam UMC location Vrije Universiteit Amsterdam, Physiology, De Boelelaan 1117, Amsterdam, Netherlands
2: Amsterdam Cardiovascular Sciences, Heart Failure & Arrhythmias, Amsterdam, The Netherlands

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy. At cellular level, HCM is characterized by mechano-energetic uncoupling, i.e. a mismatch between ATP supply and demand. Mavacamten is the first-in-class, FDA/EMA approved cardiac-specific myosin inhibitor for the treatment of adults with symptomatic obstructive hypertrophic cardiomyopathy (HCM). We here investigated the effects of acute and chronic mavacamten (MAVA) treatment on mitochondrial and contractile function in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from two patients.

Stem cells from two patients carrying the *MYBPC3* c.2373insG (p.W792fs) variant and two unrelated healthy controls were differentiated to cardiomyocytes. Acute effects of MAVA on mitochondrial function was assessed in 2D iPSC-CMs using the Seahorse analyzer and cell morphology by immunofluorescent staining. Chronic MAVA effects were studied in engineered heart tissues (EHTs), where contractility was measured by video-optical contractile analysis and mitochondrial respiration was assessed using the Oroboros.

Seahorse measurements revealed that patient-derived CMs had a reduced mitochondrial respiration compared to controls, consistent with lower mitochondria intensity assessed by TOMM20 staining. MAVA more strongly affected basal respiration and ATP production in control cells, while an increase in cell area was observed only in patient-derived cardiomyocytes. Patient-derived EHTs showed a distinct contraction profile with prolonged relaxation. MAVA reduced force production in all EHTs but the effect on relaxation dynamics was different between control and patient-derived EHTs.

These results demonstrate that patient-derived cardiomyocytes effectively model HCM-related phenotypes, providing a valuable platform for studying disease mechanisms and treatment responses. Both acute and chronic mavacamten treatment showed patient specific responses.

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Poster #36

Lmod2 Orchestrates Actin-Profilin Dynamics to Control Thin Filament Pointed-end Polymerization

Alla S. Kostyukova[1], Cristina M. Risi[2], Mason Summers[1], Carol C. Gregorio[3], Vitold E. Galkin[2]

[1]Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, Washington, United States of America

[2]Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, Virginia, United States of America

[3]Department of Medicine and Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, New York, United States of America

Striated muscle contraction relies on precise interactions between actin-based thin filaments and myosin-based thick filaments within sarcomeres. Accurate regulation of thin filament length is essential for optimal force generation, and even minor disruptions can lead to severe myopathies. It is now accepted that thin filament length is dynamically regulated by a network of actin-associated proteins acting at pointed filament ends including leiomodin and tropomodulin. In cardiac muscle, tropomodulin-1 stabilizes filaments by capping their pointed ends and preventing actin polymerization and depolymerization, whereas leiomodin-2 promotes actin nucleation and allows elongation of thin filaments, functioning as a more permissive regulator.

The leiomodin family is structurally related to the tropomodulin family, sharing two actin-binding sites (ABS1 and ABS2) and one tropomyosin-binding site. ABS2 is represented by a leucine-rich repeat (LRR) domain. Another unique element that distinguishes leiomodin-2 from tropomodulin-1 is a ~150-residue C-terminal extension that harbors three additional ABSs, one of which is a WH2 domain, and a poly-proline region located between the first two ABSs. The C-terminal extension binds to the sides of fully matured cardiac thin filaments in a Ca^{2+}-dependent manner.

Here, we demonstrate that while leiomodin-2 nucleates actin in the presence of profilin, deletion of the C-terminal extension greatly inhibits this ability. We show that the poly-proline region binds profilin, which corroborates the ability of leiomodin-2 to effectively promote polymerization of the actin-profilin complex. We used cryo-electron microscopy to elucidate the structure of the actively growing pointed ends from the actin-profilin complex. We show that leiomodin-2 coordinates three actin subunits at the end of the actin filament on one or two actin strands through its LRR domain, while its C-terminal extension interacts with actin protomers at a distance from the pointed end. The arrangement of LRR-bound actins differs from that of mature F-actin, so once polymerization is complete, LRR is expelled from the actin filament and cannot stay bound to the sides of F-actin. Our data suggest that the interaction of the profilin-binding C-terminal extension with actin may not only promote LRR-dependent stabilization of the terminal actin protomers, but also foster release of profilin from the leiomodin-2-profilin complex.

Establishing a "poison-peptide" induced murine model of truncated Titin-associated dilated cardiomyopathy.

Aditya Kanwal[1], Taekyeong Kim[1], Justin S Kolb[3], Gerrie Farman[3], Yu-Chieh Chen[2], Lisa Wren[1], Nicholas Legere[2], and Henk L. Granzier[3], J. Travis Hinson[1,2]
[1] The Jackson Laboratory for Genomic Medicine, Farmington, CT 06032, USA
[2] Cardiology Center, University of Connecticut Health Center, Farmington, CT 06030, USA
[3]Cellular and Molecular Medicine and Sarver Molecular Cardiovascular Research Program, University of Arizona, Tucson, AZ 85724, USA.

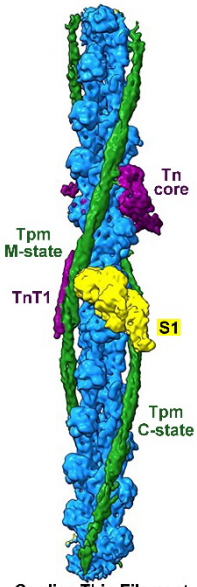
Titin truncating variants (TTNtvs) are the most common pathogenic genetic variants identified in individuals with dilated cardiomyopathy (DCM). Previous work has shown that TTNtv expression is associated with reduced levels of full-length Titin (TTN) protein, along with impaired sarcomere structure and function in affected patients. Despite their high prevalence, the pathogenic mechanisms by which TTNtvs drive disease remain incompletely understood, with ongoing debate between haploinsufficiency and poison-peptide (or dominant-negative) models of action. Investigating the role of TTNtvs in DCM has been challenging, largely due to the lack of robust in-vivo models. Although murine systems provide a valuable experimental platform for genetic manipulation, most existing models fail to develop the severe and progressive DCM phenotype observed in patients, thereby limiting mechanistic insight and therapeutic exploration.

Here, by using CRISPR technology, we demonstrate that poison-peptide mechanism is sufficient to drive dilated cardiomyopathy in a murine model harboring a TTNtv. Leveraging this novel model, we systematically interrogate the molecular, cellular and physiological consequences of TTN truncation and establish a relevant in-vivo platform for studying disease progression and evaluating therapeutic interventions.

Quantifying the Structural Basis of Thin Filament Cooperativity: The Effect of Single Myosin Heads on the Position of Tropomyosin on Actin

Olga Karpicheva, Chad Hicks, Michael J. Rynkiewicz, William Lehman.
Department of Pharmacology, Physiology & Biophysics, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA.

Comparison of cryo-EM-based atomic models of myosin-free and myosin-decorated thin filaments shows clear evidence of myosin-induced azimuthal translocation of tropomyosin to its open M-state position on actin. We observe that myosin-induced movement of semi-rigid tropomyosin spreads locally over the thin filament surface beyond the point of actin-myosin binding, thus "opening" adjacent myosin-free actin subunits for further myosin association. This behavior results in cooperative activation of the thin filament (McKillop and Geeves, 1993; Geeves and Lehrer, 1994; Vibert et al., 1997). We aim to characterize and quantify the extent of tropomyosin repositioning along cardiac thin filaments induced by single cardiac myosin-S1 heads. Previous cryo-EM-based models of myosin-decorated thin filaments were generated from filaments saturated with myosin-S1, i.e. where all actin subunits along thin filaments bound a myosin head. However, full filament decoration is non-physiological; in fact, in contracting muscle, few myosin heads attach simultaneously to neighboring actin subunits.



Cardiac Thin Filament Decorated with Single Cardiac Myosin-S1 Heads

In the current cryo-EM study, we controlled the level of myosin-S1 binding to thin filaments, then selected and analyzed filament segments where only a single myosin-S1 head bound to long stretches of otherwise S-1-bare actin subunits contained in either troponin-free (unregulated) or troponin-regulated actin-tropomyosin filaments. Each dataset yielded ~5 Å resolution 3D-reconstructions. In both cases, tropomyosin on the actin helical strand containing the bound myosin head occupied the M-state position and was twisted as in fully-decorated filaments. Here the M-state tropomyosin position extends over the TnT1-tropomyosin overlapping domain in the regulated filaments. In contrast, in both troponin-regulated and troponin-free filaments, tropomyosin, on the opposite actin strand of the filament's double-helix "untouched" by myosin, occupied the C-state position. Therefore, the myosin-induced "signal" does not appear to cross to the opposing actin strand in either filament type. Our new precision indicates that the cooperative unit size for troponin-free actin-tropomyosin is at least 5 actin subunits-long and then dissipates, whereas the cooperative unit size may vary in troponin-regulated filaments depending on myosin-S1/tropomyosin pseudo-repeat juxtaposition. We did not find a strict preference for myosin-S1 to bind to actin subunits associated with specific tropomyosin domains but do note that the troponin core-domain obstructs myosin-S1 binding at actin-tropomyosin-pseudorepeat-5.

Poster #39

Base Editing of an Upstream Open Reading Frame in *TTN* to Study Its Regulatory Mechanisms and Potential as a Therapy in DCM

Tae Kyeong Kim[1], Aditya Kanwal[1], Yu-Chieh Chen[1,2], J. Travis Hinson[1,2]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT

[2]Cardiology Center, University of Connecticut Health Center, Farmington, CT

Truncating variants in *TTN (TTNtvs)*, which encodes the sarcomeric protein titin, represent the most common genetic cause of dilated cardiomyopathy (DCM) and are also prevalent in non-genetic forms. These *TTNtvs* have been observed to result in reduced full-length titin production independent of where the mutation occurs. This haploinsufficiency mechanism likely underlies *TTN* mutation–induced DCM, highlighting the need for therapeutic approaches that restore titin protein expression levels. Prior studies indicate that untranslated regions (UTRs) within many genes contain regulatory sequences that can inhibit protein abundance via translational control mechanisms, including upstream open reading frames (uORFs) within 5′ UTRs that limit translation of the main coding sequence. However, little is known about the effect of uORFs on titin expression, their normal regulatory functions, and their capacity to be targeted in DCM to reverse haploinsufficiency.

To test our hypothesis that disrupting uORFs can relieve translational suppression of *TTN* mRNA in heterozygous TTNtv models, we designed luciferase reporter plasmids harboring mutated uORF sequence that mimic the consequence of adenine base editing and compared their activity to wildtype reporter plasmids in human iPSC-derived cardiomyocytes. Our result revealed that disruption of both uORFs increased luciferase activity through a likely translational mechanism. Notably, editing uORF2 alone was sufficient to recapitulate this effect, identifying it as the key regulatory element. To have the capacity to investigate the effect of uORF2 editing on TTN expression *in vivo*, we employed adenine base editing (ABEmax) system combined with guide RNAs specifically targeting uORF2, delivered through cardiotropic adeno-associated viruses (myoAAVs). This approach achieved ~90% editing efficiency in murine cardiomyocytes with minimal bystander edits or indels. Now that we have a tool to target uORF2, we are studying its impact on DCM in a TTNtv DCM model. Collectively, these findings establish 5′ UTR uORFs as actionable regulatory targets governing titin protein expression that require further study in DCM pathogenesis.

In Silico Prediction of Mutation Effects and Drug Efficacy on Cardiac Muscle Function

Srboljub M. Mijailovich[1], Momcilo Prodanovic [1,2], Andjela Kafedziski [1,3], Vanja Cvetkovic[1,3], Kristi Kooiker[4], Farid Moussavi-Harami[4], and Michael Regnier[5]

[1] FilamenTech Inc, Newton, MA 02458, USA,
[2] Institute for Information Technologies, University of Kragujevac, 34000 Kragujevac, Serbia,
[3]Faculty of Physics, University of Belgrade, 11000 Belgrade, Serbia,
[4]Division of Cardiology, University of Washington, Seattle, WA, USA
[5]Department of Bioengineering, University of Washington, Seattle, WA, USA

Cardiomyopathies present significant therapeutic challenges, with only a limited number of drugs developed for these indications. This complexity reflects the broad spectrum of disease phenotypes arising from different genetic mutations and their variable severity. To address this, we have been developing MUSICO, a stochastic multiscale simulation platform that integrates biochemical data from molecular-level interactions between contractile proteins, calcium ions, and small molecules with mechanical measurements at the level of fibrils, fibres, tissues, and whole organs. MUSICO enables quantitative assessment of how specific genetic mutations alter protein function and contribute to disease progression, while providing a rational framework for evaluating therapeutic strategies. A key capability is the development of translational matrices that integrate data across different experimental setups, conditions, and even species, providing clinically relevant predictions for cardiac patients. Using this approach, we investigated (i) the effect of myosin isoforms on cardiac muscle twitch in mice, rats, and humans; and (ii) the effect of variable incorporation of troponin-C mutations in thin filaments on cardiac muscle twitch contractions. As a proof of concept, MUSICO was applied to characterize the mechanism of action of the myosin inhibitor mavacamten (Camzyos™, FDA approved 2022) from biochemical data available prior to Phase 3 clinical trials. Simulations reproduced mavacamten's Ca^{2+}-dependent stabilization of the myosin super-relaxed state, predicting ~30% reduction in steady-state force at full Ca^{2+} activation and a ~50% reduction in twitch peak tension at therapeutic doses, with preservation of relaxation kinetics consistent with subsequent experimental measurements in engineered heart tissue. At the organ level, simulations predicted decreased peak LV pressures and improved stroke volume in hypertrophic cardiomyopathy, directionally consistent with the LVOT gradient reduction and improved exercise capacity subsequently observed in the Phase 3 EXPLORER-HCM trial. This constitutes the first prospective, physics-based prediction of a cardiac drug's human clinical effect from molecular kinetic parameters alone, written before Phase 3 data were unblinded. Furthermore, we quantified the mechanisms through which 2′-deoxy-ATP activates cardiac contraction, identifying a prolonged duty cycle and, in part, DRX head recruitment as key drivers, with quantitative predictions of twitch amplitude and relaxation kinetics across heart rates relevant to dilated cardiomyopathy therapy.

Structural and Functional Impact of the HCM-linked D219N Tropomyosin Mutation

Andrew Kim[1], Catherine Vasquez[2], Melissa Lynn[3], Romi Casillo[3], Jil Tardiff[3]

[1]University of Arizona Physiology, [2]Cellular and Molecular Medicine, [3]Biomedical Engineering

The cardiac thin filament (CTF) is a crucial regulator of contraction and relaxation. It couples the availability of cytoplasmic calcium to mechanical force production. Mutations in CTF proteins have been strongly associated with symptomatic cardiomyopathies. In 2014, a mutation identified in tropomyosin at residue 219 (D219N-Tm) was linked to the development of hypertrophic cardiomyopathy (HCM). Due to the proximity of this mutation to the cardiac troponin T-Tm (cTnT-Tm) overlap region, we investigated the structural effects of D219N-Tm using Time-Resolved Resonance Energy Transfer (TR-FRET). The TR-FRET data revealed a compaction at the overlap region in D219N-Tm CTF's under saturated calcium conditions. To understand how these structural changes affected downstream cardiac function, we generated a D219N-Tm transgenic mouse model. At 2 months of age, transgenic mice exhibited an increased percent fractional shortening (38.5%) compared to their non-transgenic (NTg) littermates (28.6%). These alterations in systolic function were coupled to changes in cardiac morphology. 2-month-old D219N-Tm hearts showed significantly elevated atrial-weight to heart-weight ratios (0.1504±0.006656) compared to NTg (0.1108±0.01827), suggesting that greater left-ventricular filling pressures may lead to atrial hypertrophy. To assess changes in myofilament activation, we conducted an NADH-coupled ATPase assay with transgenic myofibrils and NTg littermates at varying calcium concentrations. The ATPase rates and calcium sensitivity (pCa_{50}) were elevated in D219N-Tm myofibrils compared to NTg littermates. We then investigated whether these changes at the overlap affected cellular contractility and calcium transients. Contractility measurements revealed a reduction in departure velocity time, return velocity time, and time to peak, indicating faster rates of contraction and relaxation in D219N-Tm cardiomyocytes. The D219N-Tm cardiomyocytes also showed a significant decrease in baseline sarcomere length compared to the NTg controls, which may suggest the presence of residual cross-bridges after diastole. Future studies would utilize a 2,3-butanedione monoxime (BDM) treatment of unloaded NTg and D219N-Tm cardiomyocytes to determine whether these changes in baseline sarcomere length are due to differences in diastolic tension.

Poster #42

Modeling the effects of cTnI Ser23/24 phosphorylation and the cTnI-R145W Mutation

Rhyaan Mohran[1], Romi Castillo[1], Krishna Prasad Ghanta[2], Steven Schwartz[2], Jil C. Tardiff[1]

[1]Department of Biomedical Engineering, University of Arizona, Tucson, AZ

[2]Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ

The Arg145Trp (R145W) is a pathological variant in cTnI that has been associated with both HCM and RCM. While disease expression may be variable among individuals, a hallmark of HCM and RCM is diastolic dysfunction. At the molecular level, the thin filament governs relaxation via key regions of cTnI, which stabilize the blocked state. To investigate the impairment of relaxation at the myofilament level, we used stop-flow calcium dissociation kinetics with a fully reconstituted thin filament (CTF) system at baseline and with phosphorylation of cTnI-Ser23/24. There was no significant difference in calcium dissociation rate between R145W-cTnI and WT; however, upon phosphorylation, we observed a blunted calcium dissociation rate for R145W-cTnI phosphorylated (R145W-PH) compared to WT-PH. We utilized molecular dynamics to investigate the structural effects of the R145W-cTnI mutation at baseline and in the phosphorylated state. We created a double CTF model consisting of two adjacent regulatory units. The blocked state for the four resulting systems was compared at the following interactions: the mobile domain of cTnI (MD-TnI) to the nearby Actin, the MD-TnI to the nearby tropomyosin (TM). For the WT system, the MD-TnI makes significant electrostatic contact with TM. With phosphorylation, we observed an increase in interaction frequency between these interacting residues. For the R145W model, we observed an increase in the number and frequency of interactions compared to WT. However, electrostatic interactions in the R145W-PH system were nearly abolished. The R145W mutation exhibited a similar number and frequency of interactions to WT between the MD-TnI and actin. In the phosphorylated systems, R145W-PH showed a significantly lower number and frequency of interactions, indicating a drop-off in stability when compared to WT-PH. This decrease in stability for R145W-PH corresponds with the blunted calcium dissociation rate observed. The increase in electrostatic interactions observed with phosphorylation likely facilitates the “fly-casting” of the MD-TnI, enhancing its rate of return to the blocked state, and thus, this is likely perturbed in the R145W-PH system. In future work, we intend to expand this study to other RCM mutations in the MD-TnI to further correlate structural changes within this region to changes in CTF deactivation.

Poster #43

Danicamtiv Partially Improves Cardiac Contractility in Human Cardiomyopathic Septal Myocardium

Maicon Landim-Vieira[1], Milagro Monroy Aguirre[1], Hailey Flannagan[1], Edward O. Olaniyan[1], Aurélia Araújo Fernandes [2], Kenneth S. Campbell[3], Weikang Ma[1]

1 Department of Biology, Illinois Institute of Technology, Chicago, IL, USA

2 Department of Morphology, Federal University of Espírito Santo, Vitória, ES, Brazil

3 Department of Physiology, University of Kentucky, Lexington, KY, USA.

Length-dependent activation (LDA) is a fundamental property of cardiac muscle that contributes to the Frank–Starling mechanism. Impairment of LDA has been reported in cardiac disease and may contribute to contractile dysfunction. Danicamtiv is a small-molecule cardiac myosin activator developed to enhance systolic performance by increasing myosin interaction with actin and improving force generation. Thus, it has been investigated as a potential therapy for systolic heart failure. In the present study, we used septal myocardium obtained from explanted human hearts of patients with dilated cardiomyopathy (DCM) and non-ischemic cardiomyopathy (NICM) to determine whether the septum displays impaired LDA and whether danicamtiv can restore contractile force. Mechanical experiments were performed in permeabilized human septal muscle preparations at 30°C, at sarcomere lengths of 2.0 and 2.4 μm, in the presence of 3% dextran to better approximate physiological lattice spacing. Both DCM and NICM displayed reduced force at all levels of calcium activation compared to control. DCM samples retained a length-dependent increase in force. In contrast, NICM septal samples showed impaired LDA. Treatment with danicamtiv did not broadly restore force across activation levels, but it increased force at submaximal activation (pCa 6.1). In addition, danicamtiv increased myofilament calcium sensitivity in both DCM and NICM, consistent with enhanced contractile activation at lower calcium concentrations. Together, these findings show that septal myocardium from NICM hearts exhibits impaired LDA, whereas DCM septal myocardium preserves this regulatory mechanism. Although danicamtiv did not fully rescue force deficits across all conditions, its ability to augment force at pCa 6.1 and increase calcium sensitivity suggests that cardiac myosin activation may partially improve contractile performance in diseased human septum.

Titin is a tunable viscoelastic transmission in muscle sarcomeres that regulates energy storage and dissipation.

Kiisa Nishikawa[1], Jenna Monroy[2], and Richard J. Casler, Jr.[3]

[1]Northern Arizona University, [2]Claremont Colleges, [3]Media Lab, Massachusetts Institute of Technology

Previous studies demonstrated that active muscles store up to 56% of the energy expended in stretching them. This energy can be used to reject perturbations and overcome obstacles. However, where this energy is stored remains unclear. Recent studies suggest that titin is a tunable viscoelastic structure whose stiffness and damping depend on strain and activation and further suggest that the tunable viscoelastic properties of titin could account for the ability of muscles to harvest or dissipate energy from applied forces. To test this hypothesis, we conducted passive and active ramp stretch experiments (amplitude = 5% L_0, speed = 1 L_0/s) across the physiological length range (0.8 to 1.2 L_0) in passive and active soleus muscles from wild-type and *mdm* mice (n = 7 - 9 muscles per genotype), which vary in exon splicing of PEVK titin. We used system identification to determine the viscoelastic dynamics that best fit the ramp stretch data. Using spring and damping constants obtained from coefficients of the transfer function, we calculated time constants and linearized titin PEVK kinetics, capturing energy flow through the elements that comprise the viscoelastic structure. Data from passive and active muscles were best fit (mean R^2 = 0.986) by a viscoelastic structure comprising two Kelvin-Voigt elements in series. In passive muscles, viscoelastic parameters varied over 2-4 orders of magnitude, demonstrating a role for I-band titin. In active muscles, parameters varied over a smaller range with changes in equilibrium length suggesting engagement of a clutch. *Mdm* muscles were passively stiffer than wild-type muscles, consistent with PEVK contour lengths estimated from exon expression data. Viscoelastic elements stored elastic energy up to 26 J/kg for ~50 ms, increasing up to 150 J/kg in active muscles for up to 1.5 s. The results demonstrate that PEVK titin transmits passive and active forces from A-band to Z-disk during ramp stretch. Activation- and length-dependent stiffness and damping enable significant energy storage and recovery, completely accounting for energy storage in muscles during active stretch. We suggest that the tunable viscoelastic transmission of muscle sarcomeres reduces the energy burden and computational cost of motor control. Supported by NSF-DBI-2319710.

Poster #45

Engineering the Endogenous Cardiac Stress Response using CRISPR-Activation Technology to Create Heart Failure Resilience

Nicholas Legere[1,2], Jianlin Feng[1], Aditya Kanwal[2], Jinting Zhang[1], Lisa Wren[2], and Travis Hinson[1,2]

[1]Calhoun Cardiology Center, University of Connecticut Health Center, Farmington, CT

[2]The Jackson Laboratory for Genomic Medicine, Farmington, CT

In response to heart failure stimuli like chronic pressure-overload, cardiomyocytes mount a cell-wide stress response altering the regulation of multiple sarcomere and cytoskeletal proteins. This remodeling of the cardiomyocyte cytoarchitecture ultimately impacts cardiac pump function. However the precise effect that each modification to the proteome has on pump function, and whether the modification is considered adaptive or maladaptive under heart failure stimuli, remains largely undefined. Furthermore, the capacity of any adaptive changes to be selectively targeted for therapeutic development using CRISPR-activation (CRISPRa) technology remains mostly unexplored.

We employed a novel proximity proteomics mouse model to quantitatively define the cardiomyocyte pressure-overload stress signature *in vivo*. This model used a genetically-encoded proximity labeling enzyme, TurboID, fused to alpha-actinin 2 (encoded by *Actn2*) to label the proteome surrounding ACTN2-based structures including the Z-disc. Following trans-aortic constriction (TAC) to induce cardiac pressure-overload, we detected 112 sarcomere and cytoskeletal proteins significantly dysregulated in failing TAC hearts relative to healthy controls, with the majority (89) increasing in abundance.

To interrogate the adaptive nature of these individual changes, as well as their therapeutic potential, we selectively upregulated the expression of several candidate stress response genes identified above in a genetic mouse model of heart failure. This was accomplished using a knock-in mouse model harboring cardiomyocyte-specific CRISPRa machinery equipped with transcriptional activators VP64, p65, and Rta (VPR). Cardiac structure, function, and lifespan were measured as therapeutic readouts following upregulation and compared to non-targeting heart failure controls. Candidate genes were prioritized by their implication in human heart failure as predicted by recent genome-wide association studies (GWAS). We found that further enhancing the expression of a single target gene enriched in the endogenous stress response was sufficient to both improve cardiac structure and extend lifespan by 50% in our mouse model of heart failure. We are further exploring the human relevance of this candidate using human stem cell-derived cardiomyocyte models. Collectively, these results nominate CRISPRa technology as a potential therapeutic approach for genetic heart failure, and support the endogenous cardiomyocyte stress response as a basis for therapeutic innovation.

Predicted X-Ray Fiber Diffraction Patterns Reveal Heterogeneous Force Distribution in Skeletal and Cardiac Muscle Actin Filaments

Momcilo Prodanovic [1,2], Andjela Kafedziski [1,3], Thomas C. Irving [4] and Srboljub M. Mijailovich[1]

[1] FilamenTech Inc, Newton, MA 02458, USA,
[2] Institute for Information Technologies, University of Kragujevac, 34000 Kragujevac, Serbia,
[3] Faculty of Physics, University of Belgrade, 11000 Belgrade, Serbia,
[4] Biology Department, Illinois Institute of Technology, Chicago, IL 60616, USA

Small-angle X-ray diffraction is a powerful technique for assessing how the modulation of molecular kinetics affects functional behavior in living muscle cells. This technique provides valuable insights into structural changes during muscle contraction while simultaneously capturing physiological data, allowing direct, real-time correlation between muscle structure and function. Here, we combine MUSICO-Fiber, which simulates sarcomere contraction and local force generation, with MUSICO-X, which predicts the corresponding X-ray diffraction patterns in skeletal and cardiac muscles. Precise forward predictions of X-ray meridional intensity profiles directly link the local force generated by crossbridges with the overall force produced in muscle fibers, calibrated against experimentally observed meridional profiles to accurately characterize the relationship between molecular events and muscle tension. These simulations provide net active tension, the direct reflection of molecular processes which generate tension, which is about 30-40% higher than the observed total tension. Total observed tension reflects both the contractile force from muscle fibers and contributions of other elastic tissue components, and it is lower than in muscle fibers due to a decrease in the fraction of the cross-section area occupied by the fibers. Experimental recordings now extend to the fourth actin meridional reflection, and MUSICO-X, which incorporates the all-atom structure of actin filaments, enables prediction of molecular arrangements and diffraction patterns directly related to molecular kinetics, local force generation, and net active tension. Matching simulations to experimental data up to fourth meridional reflection reveals the mean force in actin filaments as well as the variability in force along them. MUSICO-Fiber predicts the force in each actin filament alongside the variation in force between filaments arising from the stochastic process of myosin binding, and the heterogeneity of actin forces reflecting variable actin filament lengths in cardiac and in nemaline myopathy of skeletal muscles. These heterogeneities are recorded in X-ray diffraction patterns, and their individual contributions or combinations can be precisely separated by MUSICO-X simulations. Each characteristic force population contributes a distinct diffraction pattern, and these are combined into a single pattern matching experimental observations. These actin length heterogeneities explain why high-resolution X-ray diffraction patterns are harder to obtain in cardiac than in skeletal muscle.

Porcine Cardiac Tissue Exhibits Chamber-Specific Enzymatic and Mechanical Profiles Similar to Human Hearts

Jessica Tolley, Mike DuVall, **Sarah Lehman**, Sommyr Bass, Marcus Henze, Craig Emter, Alan J Russell

Edgewise Therapeutics, Boulder, CO

Cardiovascular disease (CVD) impacts >610 million people worldwide, presenting an extraordinary burden on patients and global healthcare systems. These diseases, while diverse in their etiology, alter the contractile properties of the atria and ventricle, often leading to concomitant CVD(s) that further complicate clinical management. However, the standards of care for these diseases are largely limited to symptom palliation with minimal advancement in next-generation therapies that target molecular progression, like sarcomere-targeted therapies. This slowed innovation is, in part, due to a lack of preclinical models that recapitulate the expression profiles and function of atrial and ventricular adult human cardiomyocytes. In contrast to commonly used model systems, mature porcine hearts express similar sarcomeric protein isoforms to adult human cardiomyocytes. To evaluate the use of this model system for both atrial and ventricular function *in-vitro*, myofibrils and permeabilized fiber bundles were isolated from porcine left ventricular and right and left atrial tissues to measure the enzymatic and mechanical function. In a NADH-coupled ATPase assay, atrial myofibrils exhibited enhanced calcium-dependent ATPase activity (pCa6.0: atrial= 13.8±0.8 $ATP \cdot s^{-1}$ vs. ventricle= 7.5±0.3 $ATP \cdot s^{-1}$, $p<0.0001$) and cooperativity (atrial= 40.6±2.6 $ATP \cdot s^{-1}$ vs. ventricle= 25.7±0.6 $ATP \cdot s^{-1}$, $p=0.0006$) compared to ventricular myofibrils, likely driven by expression of fast α-myosin heavy chain vs. slow β-myosin heavy chain expressed in ventricle (α/β: atria= 95%/5%, ventricle= 0/100%). While the specific-force was indistinguishable between the two tissues in loaded, permeabilized fibers (atria= 23.7±1.8 mN/mm^2 vs. ventricle= 23.4±1.7 mN/mm^2), atrial fibers exhibited faster activation and relaxation compared to ventricular fibers (K_{act}: atria= 0.13±0.008 s^{-1} vs. ventricle= 0.09±0.007 s^{-1}, $p=0.0001$; K_{rel}: atria= 0.50±0.03 s^{-1} vs. ventricle= 0.42±0.02 s^{-1}, $p=0.022$). The increase in kinetics resulted in an enhanced force-velocity relationship in atrial fibers (atria= 12.06±2.47 mM/mm^2 vs. ventricle = 3.12±0.56 mM/mm^2, $p\leq0.05$) and increased power output relative to ventricular fibers (atria= 1.90±0.29 vs. ventricle= 0.63±0.08. $p=0.0005$), consistent with α-myosin expression. Together, these data describe a preclinical model that expresses chamber-specific gene orthologs of adult human tissue and recapitulates the in-vitro functional differences of the chambers. Utilizing tissues from these animals as a standard model system for cardiac physiology can improve translation from preclinical studies to patient care.

Poster #48

The Structure of the Native Cardiac Crossbridge in the Rigor State.

Cristina M. Risi[1], Tyler Nguyen[1], Betty Belknap[1], Howard D. White[1], Jose R. Pinto[2], P. Bryant Chase[3] and Vitold E. Galkin[1]

1. Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, VA 23507, USA
2. Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, USA
3. Department of Biological Science, Florida State University, Tallahassee, FL, USA

Cardiac contraction is driven by double-headed myosin cycling on cardiac thin filaments, where troponin-tropomyosin regulates myosin access to actin. Prior research used a single-headed myosin bound to bare actin, thereby limiting insight into coordination between myosin heads and the influence of troponin-tropomyosin on actomyosin interactions. Here, we report a high-resolution structure of the native cardiac rigor cross-bridge formed by heavy meromyosin bound to the thin filament. We show that direct communication between the two bound heads, uneven interactions between the heads and tropomyosin, and spatial constraints imposed by troponin govern myosin placement along the thin filament. Additionally, the two heads display non-equivalent motor-light chain interactions, yielding distinct lever-arm conformations indicative of asymmetric intramolecular strain. Together, these findings provide a structural framework for how the two myosin heads coordinate and how the components of the thin filament are integrated into force generation by active cross-bridges.

Intrinsic Modifier Effect of Fetal-cTnT on Disease Progression in Sarcomeric Cardiomyopathies

Lynn ML[1], Vasquez C[2], Castillo RC[1], Kim A[3], Tardiff JC[1,2,3]

[1] Dept of Biomedical Engineering, University of Arizona, Tucson, AZ
[2] Dept of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ
[3] Dept of Physiology, University of Arizona, Tucson, AZ

Human cardiomyopathies exhibit a wide range of phenotypic variability. It is thought that genetic modifiers exist that alter disease trajectory for susceptible individuals. Yet, identifying specific modifiers remains challenging. In a subset of sarcomeric cardiomyopathies, such as the DCM-causative D230N-Tm and HCM-causative R92L-cTnT, distinct age-dependent disease progressions are observed. Within the regulatory cardiac thin filament (CTF), the Tropomyosin (Tm) overlap contains interdigitating Tm dimers and the N-terminus of cardiac Troponin T (cTnT). While Tm is stably expressed throughout life, cTnT undergoes an age-dependent transition, from its fetal to its adult isoform, altering the structure of the N-terminus. Thus, we postulate that fetal-cTnT acts as an intrinsic modifier of CTF function via structural changes at the Tm-overlap. To evaluate if fetal-cTnT can alter disease trajectory we assessed cardiac function in D230N-Tm and R92L-cTnT mice bred to fetal-cTnT mice generating DNF and RLF, respectively. DNF hearts exhibited worsened systolic and diastolic function while the function of RLF hearts improved. To elucidate the mechanisms underlying this we employed ATPase, Ca^{2+} stopped-flow kinetics and differential scanning calorimetry (DSC). While fetal-cTnT alone had minimal effect, co-expression elicited independent changes in Ca^{2+} sensitivity of ATPase in isolated myofibrils and Ca^{2+} kinetics in reconstituted CTFs. DNF exhibited increased Ca^{2+} sensitivity accompanied by a decreased rate of Ca^{2+} dissociation compared to D230N-Tm alone. Meanwhile, RLF had reduced maximal rate of ATPase and an increased rate of Ca^{2+} dissociation compared to R92L-cTnT alone. DSC was used to assess changes in the thermodynamics of the CTF at the Tm-overlap. For DNF filaments we observed an additive increase in thermal cooperativity, a surrogate of reduced flexibility, at the Tm-overlap beyond that elicited by D230N-Tm alone. RLF filaments exhibited no change in flexibility at the Tm-overlap compared to R92L-cTnT. These data suggest a potent modifier effect of fetal-cTnT, capable of altering phenotypic trajectories, and may represent a targetable site of dysregulation contributing to the heterogeneity observed in the patient population.

Poster #50

A Gain-of-Function Missense Mutation in Cardiac Myosin Binding Protein-C's M-Domain Promotes the ON State of Myosin Heads in Passive Cardiac Muscle

Rachel L. Sadler[1], Nichlas Engels[2], Christine Delligatti[3], Dylan Reichert[4], Alex Noonan[3], Grace Privett[3], Katelyn Manross[3], Khoi Nguyen[3], Anthony Hessel[3,5], Samantha P. Harris[1]

1. Department of Physiology, University of Arizona, Tucson, AZ, USA. 2. Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA. 3. Accelerated Muscle Biotechnologies, Mansfield, MA, USA. 4. Department of Biomedical Engineering, University of Arizona, Tucson, AZ, USA. 5. Institute of Physiology II, University of Muenster, Germany.

Cardiac myosin binding protein-C (cMyBP-C) regulates contraction and relaxation of the heart through interactions with thin and thick filaments. Mutations in the encoding gene, *MYBPC3*, are a leading cause of hypertrophic cardiomyopathy (HCM), a condition affecting 1:500 people worldwide and characterized by left ventricular wall thickening and diastolic dysfunction. An HCM-associated mutation, L348P (L352P in humans), increases actin-binding affinity of cMyBP-C's regulatory M-domain. In heterozygous cardiomyocytes from our CRISPR-generated mouse model (L348P-CR), L348P increased calcium sensitivity and slowed relaxation, suggesting that increased M-domain affinity for actin promotes the ON state of cardiac myofilaments. Here, we tested the consequences of enhanced cMyBP-C-actin interactions on myofilament structure in L348P-CR mice via small-angle X-ray diffraction (XRD). XRD experiments were conducted in passive conditions at the Life Science X-ray Scattering Beamline at NSLS-II using detergent-permeabilized papillary bundles. L348P increased the intensity ratio of equatorial 1,1 and 1,0 reflections (indicating a shift of myosin heads away from the thick filament backbone) and increased spacing of the third-order meridional reflection (M3) (indicating an increase in the axial spacing of myosin heads). These results support our hypothesis that L348P predisposes cardiac thick filaments towards their ON state in the absence of calcium. In subsequent experiments, we incubated samples in 3% dextran to counteract non-physiological lattice spacing increases from permeabilization and raised sample temperature from room temperature to 30° C. These modifications increased image resolution and permitted measurement of higher order reflections, such as myosin layer line 4 (MLL4). MLL4 had reduced intensity in L348P samples, indicating a loss in the helical myosin order associated with the OFF state of resting muscle. With dextran and increased temperature, equatorial intensity ratios decreased in both WT and mutant samples. Furthermore, L348P's effects on equatorial intensity ratios and M3 spacing relative to WT controls were not detected in the new experimental conditions. Taken together, these results emphasize the impact of lattice spacing and/or temperature on myofilament structure. Future experiments will include cardiomyocyte mechanics assays in dextran to determine whether L348P's impact on calcium sensitivity and cell stretch responses persists with compressed lattice spacing. Supported by NIH HL080367 and GM156170.

Cardiac specific AAVs improve heart function in a novel mouse model of hypertrophic cardiomyopathy

Christopher M. McAllister[1], Kyrah L. Turner[1], Lucy S. Plenge[1], Andrea D. Thompson[2], Sharlene M. Day[1]

[1]Division of Cardiovascular Medicine, The University of Pennsylvania. [2]Cardiovascular Medicine, The University of Michigan

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular condition. Variants in the gene myosin-binding protein C3 (*MYBPC3*) are the primary cause of HCM. MyBP-C (protein encoded by *MYBPC3*) interacts with myosin and actin in the sarcomere to regulate contractility.

Both pathogenic truncating and missense variants in *MYBPC3* cause HCM. AAV9 delivery of wild-type MyBP-C has been shown to restore haploinsufficiency and correct pathogenic phenotypes in truncating iPSC-CMs and MyBP-C knockout mice. Because their molecular mechanism is poorly understood, and the lack of *in vivo* models, missense *MYBPC3* variants have received significantly less attention. However, our lab has shown that unlike truncating variants, missense variants are not haploinsufficient and the mutant protein is in the sarcomere. Because of this it is unknown if the same AAV gene replacement approaches that have been effective in correcting pathogenic phenotypes in truncating models, will also have a beneficial effect in a *MYBPC3* missense model.

First, we established a mouse model carrying the HCM causing R506W missense variant. These mice have increased LV mass, concentric hypertrophy, increased isovolumetric relaxation time, and a modestly reduced ejection fraction. Using AQUA peptides, we quantified the ratio of wild-type to mutant MyBP-C protein and determined that in a heterozygous mouse, the mutant protein represents approximately 15% of the total protein.

Next, we tested if delivery of *MYBPC3* using a novel cardiotropic AAV serotype (GTP100) with different enhancer & promoter combinations could correct the phenotype observed in the R506W mouse model.

To date we have treated mice with multiple AAVs and at three months post-injection, serval vectors have reduced LV mass, interventricular septum thickness, LV posterior wall thickness, and increased ejection fraction. In addition to improving cardiac function viral delivery of *MYBPC3* was able to suppress the mutant allele fraction from 100% to ~10% in homozygous m

Structural Dynamics of Slow Skeletal MyBP-C N-terminal Domains: Effects of Phosphorylation and Splicing in the Pro/Ala-Rich Linker

Morgan Seffrood, Thomas A. Bunch, Ph.D., Victoria C. Lepak, and Brett A. Colson, Ph.D.
Department of Cellular & Molecular Medicine, The University of Arizona, Tucson, AZ 85724

Precise regulation of sarcomeric protein interactions is essential for tuning muscle contractility, yet the structural mechanisms underlying this regulation are incompletely understood. Slow skeletal myosin binding protein-C (sMyBP-C) is a key sarcomeric regulator of muscle contractility, acting through interactions between its N-terminal domains and actin and myosin. Similar to its cardiac paralog, sMyBP-C is phosphorylated at its N-terminus in response to β-adrenergic stimulation, enhancing muscle function. However, the structural and mechanistic effects of phosphorylation on these interactions remain poorly understood. To address this, we examined the effects of phosphorylation on the sMyBP-C N-terminal fragment (sC1-C2). Cosedimentation and fluorescence resonance energy transfer (FRET)-based binding assays demonstrate that phosphorylation reduces sC1-C2 binding to actin. To define the underlying structural changes, we used time-resolved FRET (TR-FRET) to measure distances and distance distributions within the N-terminus. In the unphosphorylated state, the Pro/Ala-rich linker region (PAL) is in close proximity to the C1 domain, and C1 and C2 domains, though separated by the motif, are also in close proximity. Phosphorylation of a serine residue in the PAL induces structural expansion between sites in the PAL with increases of 4.5 ± 2.0 Å in the center distance and 4.0 ± 1.9 Å in the distribution width (FWHM). Additional phosphorylation-induced changes were observed between the PAL and C1, (Ddistance = 3.2 ± 0.4 Å; DFWHM = 5.4 ± 0.2 Å), and surprisingly, between the C1 and C2 domains (Ddistance = 4.7 ± 1.3 Å; DFWHM = 8.1 ± 1.3 Å), indicating dynamic conformations in the phosphorylated state. We also examined a splice variant that lacks 25 amino acids from the PAL, including the phosphorylation site, and exhibits altered actin thin-filament motility. Our FRET results show a small decrease in the interprobe distance in the PAL relative to the full-length sequence in the long form, suggesting the 25 amino acids form a compact loop structure. Overall, these findings establish that phosphorylation and alternative splicing alter the N-terminal conformation and dynamics of sMyBP-C. This work defines a structural basis for how phosphorylation and isoform diversity might tune muscle contractility, with broad implications for sarcomeric regulation in health and disease.

TTN Truncating Variants Associate with Increased Passive Stiffness and Regional Fibrosis in Human Non-Ischemic Cardiomyopathy

Minton AT[1,2], Wilkerson EL[2], Terry CD[2], Campbell KS[1,2]

[1]*Department of Physiology, College of Medicine, University of Kentucky*

[2]*Division of Cardiovascular Medicine, Department of Internal Medicine, University of Kentucky*

Heart failure is the leading cause of morbidity and mortality worldwide, yet the mechanisms linking genetic variation to myocardial dysfunction remain incompletely defined. Truncating variants in the titin gene (TTNtvs) are the most common inherited lesions in cardiomyopathy, but few studies integrate genomic data with myocardial structure and function in human tissue. Leveraging a cardiac biobank containing more than 20,000 specimens from 700 patients, we combine genomic, mechanical, histological, and clinical data to examine how *TTN* truncation influences myocardial remodeling.

Ultra-deep whole-exome sequencing (median depth 256×) was performed on myocardial tissue from 313 patients with advanced heart failure and 27 organ donors. Truncating alleles were stringently prioritized using established annotation metrics: CADD (>20, predicted deleteriousness), GERP++ (>2.0, evolutionary conservation), and dbscSNV-ADA (≥0.6, splice-site disruption). TTNtvs were mapped to sarcomeric domains and annotated by exon usage (percentage spliced in). Permeabilized muscle fibers from organ donors and patients with non-ischemic cardiomyopathy (TTNtv carriers and non-carriers) were used to measure force–pCa relationships. Intra- and extracellular contributions to passive stiffness were assessed using stretch protocols following incubation with high-salt relaxing solutions and quantified as Young's modulus. Fibrosis was quantified using picrosirius red staining of left-ventricular free wall and septal tissue.

TTNtvs were identified in 20 of 340 hearts (19 failing, 1 donor), comprising 14 unique alleles. Most occurred in highly expressed exons (71%) and localized to the A-band C-zone (57%), with a recurrent hotspot in meta-exon 326. Among the 19 failing hearts, 15 and 4 patients had non-ischemic and ischemic disease, respectively. Clinical features did not differ by TTNtv carrier status in ischemic cardiomyopathy but distinguished subgroups within non-ischemic cardiomyopathy. TTNtv carriers with non-ischemic cardiomyopathy were comparable in age to donors, unlike diseased non-carriers ($p<0.001$). Moreover, left-ventricular mass index and QRS duration were lower in TTNtv carriers with non-ischemic cardiomyopathy compared with diseased non-carriers (both $p<0.05$). Active contractile properties derived from Hill fits of the force–pCa relationship, including max/minimal force, calcium sensitivity (pCa_{50}), and cooperativity (n^H), were similar between donors and patients with non-ischemic cardiomyopathy (TTNtv carriers and non-carriers). In contrast, passive mechanical properties differed by stiffness component ($p<0.001$) and experimental group ($p=0.01$), with greater total Young's modulus in TTNtv carriers with non-ischemic cardiomyopathy compared with diseased non-carriers ($p<0.05$) and donors ($p<0.01$). Fibrosis was lower in septal tissue compared with paired left-ventricular free wall tissue of TTNtv carriers with non-ischemic cardiomyopathy ($p<0.001$) and septal tissue of diseased non-carriers ($p<0.01$).

TTN truncations cluster within constitutively expressed A-band exons and associate with a non-ischemic phenotype characterized by smaller ventricular size, increased passive myocardial stiffness, and reduced septal fibrosis without major changes in active contractile function. Together, these findings support a model in which TTNtvs preferentially impact passive mechanical properties rather than active contractile function in non-ischemic cardiomyopathy.

Ongoing studies will integrate transcriptomic and proteomic profiling with myocardial mechanics to define molecular pathways linking *TTN* truncation to passive stiffness and cardiac remodeling. Complementary experiments in engineered heart tissues and Sudan Black B staining of human myocardium will evaluate how altered protein turnover and proteostatic burden contribute to cardiomyopathy associated with TTNtvs.

Poster #54

Leiomodin 2 is a processive pointed-end elongator of actin filaments

Sudipta Biswas[1], Tania M. Larrinaga[2,3], Sandeep Choubey[4], Carol C. Gregorio[3,] and Shashank Shekhar[1,*]

[1] Departments of Physics, Biochemistry and Cell Biology, Emory University, Atlanta, USA

[2]Department of Cellular and Molecular Medicine and Sarver Molecular Cardiovascular Research Program, The University of Arizona, Tucson, AZ 85724, USA.

[3]Department of Medicine and Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[4]The Institute of Mathematical Sciences, Chennai, India

***Correspondence**: Shashank Shekhar (shekhar@emory.edu)

ABSTRACT

The actin cytoskeleton drives essential processes like cell migration and muscle contraction. While barbed-end polymerization is well-established, pointed-end elongation was long considered impossible *in vivo*. Here, we demonstrate that Leiomodin 2 (Lmod2), which localizes to thin-filament pointed ends (PEs) in striated muscle cells, functions as the first identified eukaryotic processive actin polymerase. Single-molecule and single-filament imaging reveal that Lmod2 stably associates with PEs *in vitro*, enabling elongation even in the presence of high profilin concentrations found in the cytoplasm that otherwise would cause depolymerization of free PEs. Lmod2's activity also persists in the presence of tropomyosin, underscoring its physiological relevance. We find that both processivity and elongation rate of Lmod are dependent on its WH2 domain. Remarkably, human dilated cardiomyopathy-associated mutations in Lmod2 greatly reduce Lmod2's PE elongation activity, providing a potential mechanism for disease progression, underscoring the essential role of its actin polymerase activity in formation and maintenance of muscle sarcomeres.

Poster #55

Disruption of Tropomyosin–Troponin T Interactions at Tropomyosin Residue Y261 Alters Thin Filament Cooperativity and Calcium Sensitivity

Alfredo Bongiorno[1], Sanjana U. Nistala[2], Michael J. Rynkiewicz[3], Balajee Ramachandran[3], William Lehman[3], Stuart Campbell[2], Jeffrey R. Moore[1]
1. Dept. of Biological Sciences, University of Massachusetts Lowell; 2. Dept. of Biomedical Engineering, Yale University; 3. Dept. of Pharmacology Physiology & Biophysics, Boston University Chobanian & Avedisian School of Medicine

Missense mutations in cardiac muscle α-tropomyosin are associated with both hypertrophic and dilated cardiomyopathies. However, the molecular mechanisms by which these mutations alter thin filament regulation remain incompletely defined. Tropomyosin (Tpm1.1) residue Y261 is of particular interest because of its potential interactions with the N-terminal region of troponin T (TnT1) at the tropomyosin overlap junction. Moreover, a Y261C substitution has been reported in a single clinical case of pediatric hypertrophic cardiomyopathy. We have therefore hypothesized that perturbations at this site could disrupt TnT1–tropomyosin interactions and alter thin filament regulation.

We engineered substitutions at Y261 of tropomyosin (Y261C, Y261E, Y261F, Y261Q) and assessed their effects on binding to thin filaments and on myofilament function. Co-sedimentation assays demonstrated that mutations lacking an aromatic side chain (Y261E, Y261Q) significantly reduced TnT1 affinity for actin–tropomyosin filaments, whereas Y261F preserved near–wild-type binding. All Y261 mutations reduced tropomyosin affinity for actin, suggesting that this residue is critical for proper Tpm1.1 end-end binding along the thin filament during binding. Indeed, MD simulations with the Y261E substitution showed altered structure of the tropomyosin overlap domain. In vitro motility assays revealed that decreased TnT1 affinity correlated with increased Ca^{2+} sensitivity, as reflected by leftward shifts in pCa50. These shifts are consistent with reducing nearest-neighbor coupling between adjacent tropomyosins, supporting a role for TnT1 in mediating cooperative interactions along the thin filament. Human engineered heart tissues (EHTs) expressing Y261C tropomyosin were generated using iPSC-derived cardiomyocytes. Y261C EHTs displayed prolonged relaxation rates, consistent with enhanced myofilament Ca^{2+} sensitivity.

Together, these results identify Y261 as a critical determinant of tropomyosin–troponin T interactions and thin filament cooperativity. Disruption of this interface destabilizes the blocked state, enhances Ca^{2+} sensitivity, and impairs relaxation, providing a mechanistic link between tropomyosin mutations and cardiomyopathic phenotypes. Notably, this interface contains a structurally defined potential binding pocket, and our preliminary studies identify small molecules that bind the TnT1–tropomyosin junction and modulate thin filament cooperativity, highlighting a potential strategy to directly target thin filament regulatory defects.

Interrogation of Skeletal Muscle Contractile Networks with Expansion Microscopy

Jonathon A.B. Smith[1], Margaret Mungai[1], T. Bradley Willingham[1], Brian Glancy[1,2]

[1]National Heart, Blood, and Lung Institute, National Institutes of Health, Bethesda, MD

[2]National Institutes of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD

Syncytial muscle fibers are the main cell type within composite skeletal muscle tissue and progress along a continuum of contractile and metabolic characteristics, from slow twitch-oxidative type-I fibers to fast twitch-more glycolytic type-II fibers. Sarcomeres represent the basic force-generating unit of skeletal muscle and form a non-linear matrix of connections unified across the entire length and breadth of a muscle fiber, facilitating transmission of active forces both parallel and perpendicular to the contraction axis. As disorganization of sarcomeres is a fundamental etiology of many muscle-related diseases, developing scalable methods for interrogation of muscle ultrastructure is paramount to deeper understandings of muscle and mammalian health. To aid such study, we have developed an expansion microscopy workflow that enables super-resolution imaging of muscle contractile networks using only conventional optical (e.g. confocal) microscopes.

Our expansion microscopy strategy utilizes a mechanically robust, swellable hydrogel that covalently anchors biomolecules within embedded specimens to the polymer-gel matrix, allowing physical, isotropic expansion of muscle with negligible distortion to native morphology. Using this approach, we routinely achieve ≥5-6-fold expansion of slow- and fast-twitch mouse muscle tissues, including cryopreserved OCT-embedded samples. Importantly, quantification of expanded contractile networks is robust, recapitulating findings from 3D FIB-SEM that branching events occur >2-times more frequently in slow-twitch compared to fast-twitch muscle fibers. Furthermore, we combined our expansion protocol with lattice light-sheet microscopy, coupling almost isotropic optical resolution with physical isotropic expansion, permitting volumetric nanoscale visualization of the myofibrillar matrix, as well as segmentation of mitochondrial networks and the sarcoplasmic reticulum.

Skeletal muscle integrity profoundly impacts physical performance and quality of life. Our expansion microscopy protocol improves effective imaging resolution >5-fold and poses an accessible method to substantially advance knowledge of not only contractile networks but also the spatial biology of muscle cells, application of which should reveal novel mechanisms underlying myopathies and skeletal muscle dysfunction.

Mutation-Dependent Responses to Cardiac Myosin Inhibitors in Hypertrophic Cardiomyopathy

Neha Nandwani[1,2], Camille Glaser[3], Seiji Yokota[1], Aminah Dawood[1,4], Anne Houdusse[3], Kathleen Ruppel[1], James Spudich[1]

[1]Dept. of Biochemistry, Stanford University (Palo Alto, CA); [2]Current address: Innovative Medicines Accelerator, Sarafan ChEM-H, Stanford University (Palo Alto, CA); [3]Curie Institute (France); [4]Current Address: University of California, Merced (Merced, CA)

Category: Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle

Abstract: Hypertrophic cardiomyopathy (HCM) is a genetic heart disease characterized by hypercontractile cardiac function. Small-molecule inhibitors that target cardiac myosin to modulate contractility are emerging therapies. Two such FDA-approved inhibitors, mavacamten and aficamten, bind distinct allosteric sites on myosin and improve outcomes in obstructive HCM. Hundreds of myosin point mutations are linked to HCM, potentially altering drug-binding sites or allosteric networks critical for inhibitor activity. We evaluated eight pathogenic myosin mutations (Y115H, R169G, F244L, I457T, E497D, V606M, P710R, and R712L) for their effects on mavacamten and aficamten potency using a purified actomyosin system. Four mutations significantly impaired mavacamten activity, increasing IC_{50} values 3-25-fold, whereas aficamten efficacy was unaffected across all variants. Crystal structures of three mutant myosins bound to aficamten reveal the structural basis for this differential response, demonstrating that aficamten can accommodate perturbations that disrupt mavacamten binding. These results provide the first direct evidence that HCM mutations can differentially modulate cardiac myosin inhibitor efficacy and highlight the importance of developing therapies with diverse mechanisms to enable precision treatment strategies for patients with distinct HCM mutations.

Mice with an expanded Titin C-zone

John E. Smith III, Jochen Gohlke, Zaynab Hourani, Justin Kolb, Robbert van der Pijl, Catherine Hoover Browne, Paola Tonino, and Henk Granzier

Department of Cellular & Molecular Medicine, College of Medicine – Tucson, The University of Arizona

Abstract: Across vertebrates Titin has evolved to have an A-band region composed of immunoglobulin and fibronectin III domains structured with super-repeat patterns specific for the distal D-zone (Ig-Fn-Fn-Ig-Fn-Fn-Fn) and the central C-zone (Ig-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn). So far, all DNA-sequenced vertebrates have 6 D-zone super-repeats and 11 C-zone super-repeats. We previously showed that deletion of the IA-junction did not reduce the length of the thick filament. However, deletion of super-repeats C1-C2 did shorten the thick filament by ~86nm (2 x 43nm) indicating Titin's role as a molecular ruler for the thick filament. To further explore aspects of the molecular ruler, exons encoding super-repeats C9-C10 were floxed (flanking loxP sites inserted) in mice that already had super-repeats C1-C2 deleted. After mating with a Cre delete strain, we isolated a strain of mice that unexpectedly expressed a larger-than-wildtype Titin protein. RNAseq analysis indicated that in addition to the original C1-C2 deletion, the C9-C10 deletion was present but also a duplication of the C3-C8 region resulting in a C-zone with 13 super-repeats – 2 more than seen in any wild-type vertebrate species. We present our initial characterization of this mouse strain.

Poster #59

Impact of a *TNNC1* missense variant on cardiac myofilament function in DCM

Paula Nieto Morales[1], Ron Chastain[1], Nichika R. Holdrum[1], Andrew Reilly[1], Vitold Galkin, P[2]. Bryant Chase[3], Jose Renato Pinto[1]

[1]Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, USA

[2]Department of Biomedical and Translational Sciences, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk VA 23507

[3]Department of Biological Science, Florida State University, Tallahassee, FL, USA

Sarcomeric gene mutations associated with dilated cardiomyopathy (DCM) are often characterized by reduced myofilament Ca^{2+} sensitivity, which impairs force generation, disrupts excitation-contraction coupling, and promotes pathological cardiac remodeling. Here, we describe a novel CRISPR/Cas9 mouse model carrying a *TNNC1* missense variant (Ile4Met) linked to pediatric DCM and examine the functional consequences of the heterozygous (Het) variant on myofilament mechanics and cellular Ca^{2+} handling. Papillary muscles from one-month-old mouse hearts were isolated to assess Ca^{2+} sensitivity of steady-state isometric force, while contractility and Ca^{2+} transients were measured in mechanically unloaded, intact adult cardiomyocytes loaded with the Ca^{2+} indicator fura-2 AM. Het preparations exhibited significantly reduced myofilament Ca^{2+} sensitivity at a sarcomere length of 2.1 μm, evidenced by a rightward shift in the pCa-tension relationship. Maximal force normalized to cross-sectional area at saturating Ca^{2+} was unchanged; however, at submaximal Ca^{2+} concentrations (pCa 6.0 and 5.8), Het preparations generated significantly less force than wild-type (WT) controls. Sinusoidal stiffness measurements revealed reduced stiffness in Het preparations across pCa values from 6.5 to 5.4, indicating fewer strongly bound cross-bridges under these conditions. Reduced myofilament Ca^{2+} sensitivity and impaired intracellular Ca^{2+} handling both contribute to the contractile dysfunction that defines DCM. Acute treatment of Het animals with Danicamtiv, a small-molecule myosin activator, improved overall cardiac performance *in vivo*. Echocardiographic analysis demonstrated that left ventricular ejection fraction (LVEF%) increased to levels comparable to control mice without affecting heart rate, and cardiac output was significantly improved in the Het mice. These findings suggest that enhancing myosin activity may compensate for impaired myofilament Ca^{2+} sensitivity and improve cardiac function in this DCM model.

Category: Myofilament-based Biology, Diseases and Therapeutics- Cardiac muscle

Poster #60

Title: sMyBP-C Is Essential for Tetanic Force Generation by Promoting and Stabilizing Cross-Bridge Formation

Taejeong Song
Department of Cell and Molecular Medicine and Sarver Heart Center, University of Arizona

Myosin binding protein-C (MyBP-C) is a thick filament associated sarcomeric protein that interacts with actin and myosin. Three paralogs, slow, fast, and cardiac MyBP-C, are expressed in a tissue-specific manner and exhibit distinct structural and functional properties. Slow MyBP-C (sMyBP-C), encoded by the *MYBPC1* gene, is expressed in both fast- and slow-twitch skeletal muscle fibers. Mutations in *MYBPC1* are linked to severe congenital myopathies, including lethal congenital contracture syndrome and distal arthrogryposis; however, the precise role of sMyBP-C in skeletal muscle contraction and relaxation remains incompletely understood.

To investigate the functional role of sMyBP-C, we generated a skeletal muscle specific *Mybpc1* knockout mouse model (C1KO; $Mybpc1^{fl/fl}MCK^{Cre}$). At three months of age, C1KO mice exhibited significantly reduced body mass and soleus muscle weight compared to wild-type (WT) littermates ($p < 0.05$). While peak isometric twitch force (P_t) was preserved, peak isometric tetanic force (P_o) was markedly reduced in C1KO muscles, with decreases of over 50% in soleus and over 20% in extensor digitorum longus (EDL) muscles ($p < 0.05$).

During low to high frequency electrical stimulation (12.5~150 Hz), WT muscles demonstrated efficient summation of twitch forces and generated stable tetanic contractions, with P_o exceeding fivefold of P_t. In contrast, C1KO muscles showed impaired force summation, reaching peak force after fewer stimuli and exhibiting premature force decline during sustained stimulation. Consequently, P_o in C1KO muscles was less than fourfold of P_t. Additionally, soleus muscles from C1KO mice displayed pronounced oscillations during mid-frequency stimulation (50 and 75 Hz) and a loss of the early slow phase of isometric relaxation following high-frequency stimulation.

These findings reveal a previously unrecognized functional role of sMyBP-C in stabilizing cross-bridge formation and maintaining force during repetitive stimulation. Our data suggest that sMyBP-C contributes to the temporal regulation of actomyosin interactions, promoting effective twitch summation and sustained tetanic force. This work identifies sMyBP-C as a critical modulator of skeletal muscle contractile dynamics and provides new mechanistic insight into how *MYBPC1* mutations can lead to structure and functional impairments in congenital myopathies.

Poster #61

Paradoxical Features of Hypertrophic Cardiomyopathy Mutants are Resolved by Single Molecule Optical Trap Studies on Cardiac Actomyosin

Robert C. Cail, Aaron Snoberger, Michael S. Woody, Michael J. Greenberg, Bipasha Barua, Henry Shuman, Eva Forgacs, Donald A Winkelmann, E. Michael Ostap, and Yale E. Goldman

Department of Physiology, University of Pennsylvania,
Departments of Pharmacology and of Molecular and Cellular Biology,
University of California at Davis

Heart failure due to aging, anoxia and cardiomyopathies is a leading worldwide cause of morbidity. Single molecule biophysical experiments, including optical trap (OT) studies on actomyosin have advanced understanding of heart failure. Omecamtiv mecarbil (OM) is a cardiac inotrope that led to modest improvement of heart failure in clinical trials. Surprisingly, it suppresses velocity in an *in vitro* gliding filament assay. In three-bead OT studies with the β-cardiac myosin motor, we discovered that OM suppresses myosin's working stroke and the normal dependence of actomyosin attachment lifetime on mechanical force and [ATP]. The increase in whole-heart force output in the presence of OM is explained by cooperative thin-filament activation by OM-inhibited myosin molecules, an unanticipated route to muscle activation. R712L is a cardiac myosin mutation that causes severe Hypertrophic CardioMyopathy (HCM). In the optical trap, the working stroke of R712L-myosin is reduced 4-fold. Actin attachment durations are normal. R712 is adjacent to the binding site of OM. Remarkably, OM reversibly rescues R712L's working stroke. The R712L mutation uncouples lever arm rotation from ATPase activity and this inhibition is rescued by OM. M493I is another highly penetrant HCM cardiac myosin mutation. The ADP dissociation rate, which is the rate-limiting kinetic step for actomyosin dissociation, is 5-fold slower in M493I myosin than in wild type. Actin reattachment of M493I myosin is 2-fold faster than WT. The equilibrium between the waiting SRX (super-relaxed) state, and free/disordered (DRX) myosin heads is altered by these kinetic changes, providing insight into the pathological dysfunction. These studies unravel uncertainties about the etiology of heart failure and may guide future development of therapeutic drugs.

Poster #62

INSECT INDIRECT FLIGHT MUSCLE AND VERTEBRATE STRIATED MUSCLE – TWO SOLUTIONS TO THICK FILAMENT ACTIVATION AND ENDOTHERMY.

Kenneth A. Taylor[1], Jose Pinto[2] and Pouria Gholami-Tilko[1]

[1]Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-4380

[2]Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, 32306-4300.

Striated muscle myosin filaments of vertebrates and invertebrates are very different. Those from vertebrates are more similar to each other than to any known invertebrate thick filament. Vertebrate skeletal and cardiac muscle myosin filaments are structurally homogeneous having identical lengths, determined by the protein titin, 3-fold rotational symmetry and three A-band zones, proximal or P-zone, central or C-zone and distal or D-zone. Structural differences arise mostly from different isoforms of the accessory proteins. Recently, we showed that myosin filaments from rabbit psoas muscle, a fast-twitch skeletal muscle, are highly similar to those from mouse and human cardiac muscle, differing primarily in the positioning of domains C-5 and C-6 of Myosin Binding protein-C vis-à-vis titin. These three vertebrate species diverged from a common ancestor ~80 Million years ago (Mya). Fishes, whose myosin filaments are similar to those of mammals by X-ray fiber diffraction and by negative stain electron microscopy, appeared in the Cambrian period ~525 Mya. The vertebrate thick filament structure is both ancient and adaptable to the differing roles of skeletal and cardiac muscle as well as species size. Conversely, invertebrate striated muscle myosin filaments are highly heterogeneous in multiple ways even within a single organism. Thick filament structures from the highly specialized insect indirect flight muscle (IFM) also differ across three of the four insect orders that evolved this muscle type from a common ancestor that appeared 374 Mya. Arguably, each new insect species possessing IFM needed to evolve a different thick filament for flight if its thoracic cuticle evolved different mechanical properties. To support the high wing-beat frequencies, insects possessing IFM, as well as some with direct flight muscles with high wing beat frequencies, have evolved ways to raise their thoracic temperature to 40°C or even higher to take advantage of the higher ATPase rates, and do so without cooking their brains or abdomens. The myosin filaments from indirect flight muscle highlight different solutions to the same problems, control of force and endothermy. Vertebrate thick filaments, possessing in so far as is known, a single architecture, are constructed to vary the proportions of activated and inhibited myosin heads even when relaxed, presumably as a contribution to the organisms endothermy. The generally exothermic insects require a localized, thoracic endothermy for flight which might be needed occasionally, as for the giant waterbug, or continuously, as for the fruit fly or bumble bee. IFM is poised for narrowly defined, high frequency, stretch activated contractions while maintaining thoracic endothermy. In mammals, endothermy is a continuous problem, the solution for which is contributed in part by skeletal muscle, which constitutes 40% of body mass. Supported by NIH.

Poster #63

Thick Filament Molecular Interfaces Play a Critical Role in the Pathogenesis of Hypertrophic Cardiomyopathy

Debabrata Dutta[1*+], Yuri Kim[2], Carolyn Ho[2], Jonathan G. Seidman[3], Christine E. Seidman[2,3], Roger Craig[1], and Raúl Padrón[1*].

[1]Division of Cell Biology and Imaging, Department of Radiology, University of Massachusetts Chan Medical School, Worcester, Massachusetts, USA; [2]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA, USA; [3]Department of Genetics, Harvard Medical School, Boston, MA, USA. [+]Current address: Imperial College London, London, UK

Hypertrophic cardiomyopathy (HCM) variants in genes encoding the myosin heavy chain (*MYH7*), myosin light chains (*MYL2* and *MYL3*), and cardiac myosin binding protein-C (cMyBP-C, *MYBPC3*) lead to cardiac hypertrophy, with abnormal contractility, relaxation, and energy consumption. Here, we defined the structural consequences of pathogenic and benign missense variants in these genes by mapping 233 variants (*MYH7*, n=175; *MYBPC3*, n=41; *MYL2*, n=12; *MYL3*, n=5) onto a cryo-EM-based atomic model of the human cardiac thick filament. We identified HCM variants residing in 30 molecular interfaces of the complex thick filament interactome, including the two main interfaces of the myosin interacting-heads motif (IHM), and interfaces involving the myosin heavy chain, essential and regulatory light chains, and cMyBP-C. None of the 21 variants classified as benign were within interfaces. We demonstrated earlier disease onset and adverse outcomes in HCM patients with pathogenic variants within versus outside of molecular interfaces, emphasizing their importance in normal thick filament function and improving risk stratification of patients.

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Thin Filament–MARP1–N2A Tethering Modulates Titin-Based Mechanics and Links to Contractile Dysfunction

Robbert van der Pijl[1], Stefan Conijn[1,2], Zaynab Hourani[1], Mei Methawasin[1], Coen Ottenheijm[1,2], Henk Granzier[1]

Affiliations:

1. University of Arizona, Tucson, United States of America
2. Amsterdam UMC, location Vumc, Amsterdam, Netherlands

Titin is a key regulator of passive tension through isoform composition and post-translational modification. In striated muscle, the N2A element–containing titin isoform predominates in skeletal muscle and is present in the compliant cardiac N2BA isoform. Recent work identified a mechanism in which Muscle Ankyrin Repeat Protein 1 (MARP1) tethers titin's N2A element to the actin thin filament, sequestering part of the I-band spring and increasing passive tension. To define the role of the N2A element in MARP1-dependent regulation of titin mechanics, we generated mice lacking the MARP1-binding site on titin ($Ttn^{\Delta I80–I81}$). Passive tension was measured in isolated skeletal muscle myofibrils (tibialis), enabling direct assessment of N2A-dependent effects. Baseline passive tension was unchanged in $Ttn^{\Delta I80–I81}$ myofibrils compared with wild-type; however, recombinant MARP1 increased passive tension by ~70% in wild-type myofibrils but had no effect in $Ttn^{\Delta I80–I81}$ preparations, demonstrating that MARP1-dependent stiffening requires the N2A element. In vivo, $Ttn^{\Delta I80–I81}$ mice developed age-dependent systolic dysfunction and ventricular atrophy, accompanied by reduced MARP1 protein levels, indicating that the N2A element stabilizes MARP1. Skeletal muscle similarly exhibited gastrocnemius atrophy with a modest increase in specific force. Titin isoform analysis revealed coordinated but tissue-specific adaptations, with increased N2BA:N2B ratio in left ventricle, consistent with greater compliance, and decreased N2A1:N2A2 ratio in gastrocnemius, consistent with reduced compliance. Together, these findings establish thin filament–MARP1–N2A tethering as a mechanism that modulates titin-based mechanics and links disruption of this interaction to contractile dysfunction.

Poster #65

Leiomodin 2 Mutation-Induced mRNA Decay and Protein Loss Can Be Reversed by Steric-Blocking Oligonucleotides

Christopher T. Pappas[1], Rachel M. Mayfield[1], Ava E. Dickerson[1], Lei Mi-Mi[1] and Carol C. Gregorio[2]

1. University of Arizona, Department of Cellular and Molecular Medicine, Sarver Heart Center, Tucson, AZ, USA
2. Department of Medicine and Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York City, New York, United States of America

Leiomodin 2 (Lmod2), a cardiac-predominant actin-binding protein, is required for proper thin filament assembly and contractile function. Both knockout of *Lmod2* in mice and recessive loss-of-function mutations in *LMOD2* in humans cause severe neonatal dilated cardiomyopathy. While most disease-associated variants are expected to produce truncated proteins, mutant protein is rarely detected in the hearts of patients. We show that nonsense-mediated mRNA decay (NMD) degrades transcripts that contain disease-causing *LMOD2* mutations, explaining the absence of protein. To restore mutant LMOD2 protein expression, we designed steric-blocking oligonucleotides that interfere with exon junction complex deposition, thereby suppressing NMD and stabilizing mutant mRNAs. Functional studies revealed that expression of a clinically relevant truncation mutant (Lmod2[W405*]) in *Lmod2* knockout mice attenuates progression of cardiomyopathy, demonstrating that the truncated protein retains partial function. However, homozygous Lmod2[W405*] mice develop dilated hearts with shortened thin filaments and impaired contractility, establishing that Lmod2[W405*] is hypomorphic and insufficient to sustain normal sarcomere architecture and heart function over the long term. These results identify NMD as a key driver of mutant LMOD2 protein loss, demonstrate that steric-blocking oligonucleotides can restore its expression, and suggest that even partially functional LMOD2 variants may confer therapeutic benefit in LMOD2-associated cardiomyopathy.

Loss of Novex3 Titin Alters Muscle Function and Reveals a Link Between Sarcomere Organization and Metabolic Regulation

Robbert van der Pijl[1], Dalma Kellermayer[1], Justin Kolb[1], Paola Tonino[1], Balasz Kiss[1], Jochen Gohlke[1], John Smith 3rd [1], Henk Granzier[1]

Affiliations:

3. University of Arizona, Tucson, United States of America

Alternative splicing of the titin gene (Ttn) generates isoforms that contribute to passive tension and sarcomere function in striated muscle. One low-abundance isoform incorporates exon 48, producing a truncated ~638 kDa titin protein with a unique carboxy-terminus (Novex3; Nvx3), previously implicated in calcium handling and cardiomyocyte development. Here, we investigated the role of Nvx3 in skeletal muscle function. Using a knockout mouse lacking Ttn exon 48 ($Ttn^{\Delta Nvx3}$), we found that Nvx3 is developmentally regulated, with high expression during embryonic and early postnatal stages across muscle types, followed by reduced but detectable expression in adult cardiac and fast-twitch skeletal muscle (~1/14 titin molecules), and lower expression in slow-twitch soleus (~1/40). Functional analysis revealed muscle-type–specific effects of Nvx3 deletion. In soleus, Nvx3 loss reduced force-frequency responses, induced a rightward shift, and slowed relaxation kinetics, whereas in EDL muscle, force-frequency responses were enhanced but relaxation was similarly prolonged. These changes were accompanied by reduced muscle mass in multiple fast muscles at 6 months, indicating early atrophy. Immunoelectron and super-resolution microscopy localized Nvx3 primarily to the I-band in a sarcomere length–dependent manner, with additional localization at the myofibril periphery. Despite largely preserved sarcomere ultrastructure, $Ttn^{\Delta Nvx3}$ muscles exhibited regions of hypercontracted sarcomeres, Z-disk streaming, and increased autolytic mitochondria. Transcriptomic analysis of EDL and diaphragm revealed downregulation of mitochondrial pathways, consistent with altered metabolic capacity. Together with ultrastructural evidence of mitochondrial abnormalities, these findings suggest that Nvx3 may contribute to muscle function by linking sarcomere organization to mitochondrial and metabolic regulation.

Sarcomeric Human Cardiomyopathy Registry data reveals differences in LVEFs and adverse outcome rates in patients with truncating vs non-truncating variants in *MYBPC3*.

Trisha Phan, B.S., Sharlene Day, M.D.
Cardiovascular Institute at the University of Pennsylvania

Pathogenic variants in cardiac myosin binding protein C (*MYBPC3*) are the most common cause of familial hypertrophic cardiomyopathy (HCM). We have shown previously that patients with HCM due to non-truncating pathogenic variants are diagnosed at a younger age than patients with truncating variants but otherwise exhibited similar clinical characteristics and adverse event rates. Statistical power was limited by a relatively small number of patients with pathogenic variants in the Sarcomeric Human Cardiomyopathy Registry (SHaRe) at the time (N=1,238). The current study, now with 1,947 patients with pathogenic MYBPC3 variants, sought out to find additional differences.

Mechanistically, non-truncating variants in the C10 domain behave similarly to truncating variants in that the mutant protein is highly unstable and does not localize to the sarcomere. Therefore, we included C10 missense variants with the truncating variants. Clinical data were analyzed using t-tests to compare two groups or chi-squared tests to compare two categorical variables. Kernel density estimations were used to map the distribution of echocardiographic data. Time-to-event analysis from the time of birth was performed using the Kaplan-Meier method.

Unlike in the prior study in which there was no difference in mean left ventricular ejection fraction (LVEF) between the groups, we observed that mean LVEF was significantly lower in patients with non-truncating versus truncating variants (63% versus 65%, P=0.004). The LVEF distribution was shifted significantly leftward, with a greater proportion of patients with non-truncating variants having LVEFs <60% (27% versus 17%, P=0.0001) and a lesser proportion having LVEFs >70% (27% versus 40%, P=0.0002). This finding has therapeutic implications, including consideration of genotype for ongoing and future clinical trials of cardiac myosin inhibitors. Additionally, patients with truncating variants had a higher rate of adverse outcomes compared to patients with non-truncating variants (P=0.03). However, there was a trend toward a higher proportion of cardiovascular deaths in patients with truncating variants, suggesting competing risks of death in this group of patients with an older mean age at the time of HCM diagnosis. Further experimental studies are needed to discern the mechanistic differences between non-truncating versus truncating variants in MYBPC3 to account for these clinical findings.

Investigating Cardiac Myosin Binding Protein-C-Mediated Thick Filament Regulation Using *In Silico* and *In Vitro* Methods

Tristan W. Wasley[1,2], Adriana Billante[3], Matthew C. Childers[1,2], Thomas Kampourakis[4], and Michael Regnier[1,2], Bertrand C.W. Tanner[2,5]

[1]Department of Bioengineering, College of Engineering & UW Medicine, University of Washington, Seattle, Washington.
[2]Center for Translational Muscle Research, University of Washington, Seattle, Washington.
[3]School of Molecular Biosciences, Washington State University, Pullman, Washington.
[4]Division of Cardiovascular Medicine, Internal Medicine, College of Medicine, University of Kentucky, Lexington KY 40536.
[5]Department of Integrative Physiology & Neuroscience, Washington State University, Pullman, Washington.

Hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death in young adults. The most common causes of familial HCM include mutations in *MYH7* and *MYBPC3*, encoding myosin and cardiac myosin binding protein-C (cMyBP-C), respectively. Myosin regulatory light chain (RLC) and cMyBP-C play complementary roles in fine-tuning thick-filament regulation and contractile dynamics. Specifically, RLC stabilizes the myosin lever arm and modulates the proximity of myosin heads to actin, while cMyBP-C interacts with both myosin and actin through its N-terminal domains to regulate cross-bridge recruitment. However, the molecular basis by which these proteins interact remains poorly defined. To investigate potential interactions at atomic resolution, we constructed a full-length molecular model of cMyBP-C using a combination of X-ray, NMR, and AlphaFold models. We then performed molecular dynamics (MD) simulations on individual structured N-terminal domains: C1 (154-258), trihelix bundle (319-357), and C2 (363-453). Structural ensembles generated from these MD trajectories were incorporated into docking experiments with the interacting heads motif model of myosin using the program HADDOCK. Proteins were docked using both unrestrained and restrained methods to identify interaction hotspots. Preliminary results highlight the RLC/S2 tail region of myosin and a cluster of residues on C1 (Lys195, Trp196) as potential interacting surfaces. For the C2, His397-Lys380, Lys450-Glu451 and surrounding residues showed frequent contacts with myosin S2. The trihelix bundle docking results suggest that a combination of charged and hydrophobic interactions (Arg357, Trp322, etc.) may mediate interactions with a portion of the S2 arm. In parallel, we expressed and purified recombinant human proteins bearing an N-terminal poly-His tag, including the N-terminal domains of cMyBP-C (C0-C2) and a myosin subfragment containing the RLC/S2 tail region (RLC-miniHMM). These constructs were selectively immobilized using Ni-NTA capture assays to assess binding between the two proteins. Using RLC-miniHMM as the bait protein, we observed coelution with C0C2, supporting their interaction. This integrative approach combining computational modeling and biochemical validation aims to define the molecular interface between cMyBP-C and myosin. By linking structural dynamics to function, we seek to clarify how the disruption of cMyBP-C function drives hypercontractility and informs the identification of domain-specific therapeutic targets.

Poster #69

Deletion of the Z-disk protein Filamin C dysregulates sarcomere length-dependent myofilament structure in resting cardiac muscle

Elizabeth F. Johnston[1], Zhiyang Xue[2], Kristina B. Kooiker[2], Christian Mandrycky[3], Timothy S. McMillen[3], Weikang Ma[4], Thomas C. Irving[4], Michael Regnier[3,5,6,7], and Joseph D. Powers[1,2,5,6,7]

[1]Department of Laboratory Medicine & Pathology, University of Washington, Seattle, WA, US
[2]Department of Mechanical Engineering, University of Washington, Seattle, WA, US
[3]Department of Bioengineering, University of Washington, Seattle, WA, US
[4]Center for Synchrotron Radiation Research and Instrumentation, Illinois Institute of Technology, Chicago, IL, US
[5]Center for Translational Muscle Research, University of Washington, Seattle, WA, US
[6]Center for Cardiovascular Biology, University of Washington, Seattle, WA, US
[7]Institute for Stem Cell & Regenerative Medicine, University of Washington, Seattle, WA, US

Dilated cardiomyopathy (DCM) is a common and deadly form of heart disease often associated with mutations in genes encoding sarcomere or cytoskeleton proteins that confer cardiomyocyte hypocontractility, eccentric hypertrophy, and subcellular remodeling. The cytoskeletal protein Filamin C (FLNC) interacts with multiple proteins in the Z-disk and the costamere, suggesting that it contributes to Z-disk stability and mechanical force transmission in cardiomyocytes. Numerous FLNC variants have been found in DCM patients, but the underlying mechanisms driving DCM remodeling and hypocontractility in these patients are poorly understood. To investigate this, we employ inducible and cardiac-specific FLNC-knockout (FLNC-KO) mouse models of DCM in an array of structural and biomechanical analyses. By 2 weeks post-FLNC silencing in adult mice, echocardiography revealed significantly reduced ejection fraction and increased ventricular diameter in both homozygous and heterozygous FLNC-KO hearts. Single cardiomyocytes isolated from FLNC-KO hearts exhibit significant myofibrillar disarray, hypocontractility, and eccentric hypertrophy compared with controls, as we have shown previously. Here, we performed small-angle X-ray diffraction analysis using resting demembranated cardiac preparations from control and FLNC-KO hearts at short (2.1μm) and long (2.3μm) sarcomere lengths (SL) to test whether FLNC deletion affects the SL-dependence of myofilament structure in the heart. At each SL, we find that myofilament lattice spacing ($d_{1,0}$) is compressed in FLNC-KO preparations compared with controls, while the average radial position of myosin heads ($I_{1,1}/I_{1,0}$) is not different in FLNC-KO preparations compared with controls. Longitudinal spacing of myosin heads along the thick filament (S_{M3}) increases upon stretch only in FLNC-KO preparations, while the periodic order (I_{M3}) decreases upon stretch for both control and, to a greater extent, FLNC-KO preparations. Finally, the spacing of periodic elements on the thick filament backbone (S_{M6}) increases in control preparations from SL 2.1 to 2.3μm, but it does not change upon stretch in FLNC-KO preparations. Our results suggest that a loss of FLNC alters the SL-dependence of myofilament structure in the heart. In ongoing work, spatially explicit computational models will be developed together with *ex-vivo* biomechanics assays to further investigate the relationship between multiscale structural remodeling, mechanical properties of distinct subcellular domains, and contractility in hearts with FLNC-based DCM. (350/350 words)

Structural Dynamics of the Autoinhibited Interacting Heads Motif in Human Beta-Cardiac Myosin

Jinghua Ge[1], Michael R. Ebert[1], Andrew McNaughton[1], Shane R. Nelson[2], Neela Yennawar[1], David M. Warshaw[2] and Christopher M. Yengo[1]

[1]Department of Cell and Biological Systems, Pennsylvania State University College of Medicine, Hershey, PA 17033

[2]Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, VT 05405

Thick filament regulation in cardiac, skeletal, and smooth muscle is known to involve formation of the interacting heads motif (IHM), an evolutionarily conserved conformation of myosin with the heads folded back on the tail and interacting with each other. The IHM effectively turns off myosin because the heads are prevented from interacting with actin and thus unable to generate force. In addition, the IHM is thought to be associated with the super-relaxed state (SRX) with 5-10 fold slower basal ATPase activity, which is thought to be an important energy conservation mechanism in the heart. However, there is a controversy in the muscle field about the correlation between the SRX and IHM. We have utilized small angle x-ray scattering (SAXS), two FRET biosensors, and transient kinetic methods to examine the correlation between the SRX and IHM in purified human beta-cardiac myosin containing 15 heptads of the coiled-coil (M2β-15 HP). Equilibrium SAXS measurements suggest that M2β-15 HP adopts the IHM in a salt-dependent manner and the fraction in the IHM can be quantified using structural modeling. A dilated cardiomyopathy (DCM) mutant (E525K) stabilizes the IHM, especially at high salt concentrations, which correlates well with our steady-state FRET measurements. Transient kinetic measurements suggest the FRET change associated with IHM formation following ATP binding is rapid (≥5 s^{-1}) at low salt. We also examined the FRET change upon mixing from a low salt to a high salt condition (salt-jump) to monitor the transition out of the IHM and found it to be extremely rapid (≥1000 s^{-1}). Our results fit a model in which the ATPase activity is slowed (SRX state) even while myosin rapidly fluctuates between the IHM and other more Open conformations. The physiological advantage of rapidly transitioning out of the IHM is that shifting myosin heads from an off- to an on-state would not be rate-limiting for length-dependent muscle activation.

Left Ventricular Myocyte Structure and Mechanics in Transthyretin Amyloidosis Cardiomyopathy

Marcus Rhodehamel[1], Vivek P. Jani[1], Atharva Mulay[1],
Weikang Ma[2], David A. Kass[1], Mark J. Ranek[1]
[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[2]BioCAT, Department of Biological Sciences, Illinois Institute of Technology, Lemont, Illinois, USA

Introduction: Transthyretin (TTR) is a homotetrameric protein that transports thyroxine and retinol-binding protein in the bloodstream. Destabilization of TTR into monomers can lead to amyloidogenic aggregation that, when accumulated in the heart, causes amyloid TTR cardiomyopathy (ATTR-CM), a restrictive heart disease characterized by reduced exercise capacity and diastolic dysfunction. Prior work on multicellular myocardial strips from ATTR-CM patients found reduced calcium-activated contractility, attributed to extracellular matrix expansion via fibrosis and amyloid deposition. However, whether ATTR-CM impairs intrinsic cardiomyocyte function independent of the extracellular matrix remains unknown. Here, we report the biomechanical analysis of demembranated left ventricular (LV) cardiomyocytes from ATTR-CM patient biopsies versus non-failing (NF) controls, and tested the potential for protein kinase G (PKG) to modify these properties given its previously reported benefits on diastolic dysfunction.

Methods: Demembranated LV cardiomyocytes from ATTR-CM (n=8) and NF controls (n=12) were assayed for structural and mechanical properties with and without cyclic-GMP-stimulated recombinant PKG.

Results: ATTR-CM myocytes had reduced maximum tension generation (12.5±3.1 vs 23.1±5.1 mN/mm², $p=2.5e^{-20}$), increased calcium sensitivity (EC50: 1.46±0.43 vs 1.94±0.46 μM[Ca^{2+}], $p=1.3e^{-6}$), and unchanged cooperativity (n_H: p=0.1) versus NF. Maximal power was also reduced (49.3±20.3 vs 84.3±32.3 mW, $p=3.9e^{-4}$). Crossbridge detachment rate was slower (18.4±8.1 vs 27.0±7.9 s^{-1}, $p=1.5e^{-4}$) with borderline faster attachment rate (0.94±0.45 vs 1.17±0.47 s^{-1}, p=0.06) and prolonged stress relaxation time (1.7±0.7 vs 1.3±0.5 s, p=0.04). X-ray diffraction showed decreased $I_{1,1}/I_{1,0}$ ratio (0.11±0.06 vs 0.26±0.1, $p=7.4e^{-12}$) and lattice compression (38.5±1.19 vs 40±2.0, $p=4.2e^{-5}$). Resting tension was unaltered (p=0.12). PKG incubation raised maximum tension in ATTR-CM by 14% ($p=5.3e^{-5}$) without affecting NF controls (p=0.41). Calcium sensitivity (p>0.1), cooperativity (p>0.4), and resting tension (p>0.18) were unchanged post-PKG.

Conclusions: LV cardiomyocytes from ATTR-CM patients have depressed activated tension, increased calcium sensitivity, reduced power output, reduced thick filament activation, and delayed relaxation mechanics. These findings reveal pathological changes in myocyte structure and function also contribute to systolic dysfunction in ATTR-CM in addition to amyloid fibril deposition in the extracellular matrix. The selective increase in activated tension without altered resting tension following PKG suggests a potential therapeutic strategy for systolic dysfunction in ATTR-CM, though it does not address diastolic dysfunction.

Cardiac troponin I tyrosine phosphorylation differentially regulates myofilament function.

Helena Zanella, MS[1]; Ali Ulker, BS; Elizabeth Brundage, BS; Huan He, PhD; Jose Pinto, PhD; Jonathan Davis, PhD; Brandon Biesiadecki, PhD.

1. The Department of Physiology and Cell Biology, Davis Heart and Lung Research Institute, College of Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, USA.
2. Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA.
3. Translational Science Laboratory, College of Medicine, Florida State University, Tallahassee, FL, USA.
4. Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, USA.

To maintain health, the heart matches its contraction and relaxation to meet the body's demand by kinase mediated serine/threonine phosphorylation. Central in this modulation is the serine phosphorylation of the cardiac myofilament troponin inhibitory subunit (cTnI). In addition to serine and threonine phosphorylation, tyrosine phosphorylation is present in the heart, however its role to directly modulate myofilament contractile function is not fully understood. We previously demonstrated cTnI undergoes Src-mediated phosphorylation at tyrosine 26 (Y26) to decrease myocardial calcium sensitivity and accelerate *in vivo* cardiac relaxation. In addition to cTnI Y26, proteomics have demonstrated cTnI tyrosine 29 (Y29) and 112 (Y112) are phosphorylated in the mouse heart. We hypothesized that the phosphorylation of cTnI at Y29 and Y112 are significant to modulate cardiac function. To investigate if Y29 and Y112 are targets for Src-mediated phosphorylation, we treated human cTnI WT and cardiac troponin (cTn) with Src. Phos-tag Western blot demonstrated three cTnI phosphorylated bands, indicating Src mediated phosphorylation of all three cTnI tyrosine residues. To determine the effect of cTnI Y29 and Y112 phosphorylation on cardiac muscle contractile function, we measured calcium regulated force of skinned rat trabeculae following exchange with recombinant human cTn containing unphosphorylated (WT), Y26E, Y29E or Y112E pseudo-phosphorylated cTnI. Trabeculae exchanged with cTn containing Y26E, Y29E or Y112E all similarly decreased calcium sensitive force compared to cTn WT exchange. To investigate the mechanism responsible for this cTnI tyrosine phosphorylation force effect, we measured the rate of calcium dissociation from IAANS-labeled troponin C (TnC) by stopped-flow. Thin filaments containing cTnI Y26E, Y29E or Y112E all differentially increased the rate of calcium dissociation compared to WT filaments. We next measured the rate of calcium association to IAANS-labeled TnC. Reconstituted thin filaments were subjected to artificial calcium transients of varied concentrations by stopped-flow and the transient occupancy of calcium binding to TnC measured. Only thin filaments containing cTnI Y112E decreased the calcium-TnC transient occupancy indicating decreased calcium association to TnC. These results support that the tyrosine phosphorylation of cTnI at Y26E, Y29 or Y112E differentially regulate cardiac muscle function and establish new potential targets to modulate cardiac relaxation.

Myosin S2 in Cardiac Contractility and Hypertrophic Cardiomyopathy Pathogenesis

Steph Rzewnicki, Kalyani Ananthamohan, Zachary T. LaRochelle, Brent Cernyar, Henry M. Gong, Ayman Farag, Jonathan Kirk, Michael J. Previs, TaeJeong Song, Hesham Sadek, and Sakthivel Sadayappan

Abstract:

Background: Hypertrophic cardiomyopathy (HCM) is a common inherited cardiac disease that affects approximately 1 in 200 individuals. Mutations in the cardiac β-myosin heavy chain (*MYH7*), the predominant adult myosin isoform expressed in the left ventricle, are strongly associated with HCM development. Pathogenic variants localize to the myosin head and the initial 126 amino acids of the tail (proximal S2). Previous studies largely focused on the functional consequences of mutations in the myosin head, whereas comparatively less attention has been given to variants in proximal S2. It is known that proximal S2 variants reduce modulatory protein binding, however, the molecular consequences leading to hypercontractility and HCM have not been described.

Methods: To investigate the impact of clinical variants in the proximal S2 region of α-myosin, a novel CRISPR–Cas9–mediated knock-in mouse model (*Myh6*$^{E932\Delta}$) harboring a mutation homologous to the human pathogenic variant *MYH7*$^{E930\Delta}$ was generated, and its functional consequences were evaluated at the whole-heart, myofibrillar, and cardiomyocyte levels. Heterozygous (Het) mutant (*Myh6*$^{E932\Delta}$) mice were compared to wild-type (WT) littermates in the present study.

Results: By 9 months of age, Het *Myh6*$^{E932\Delta}$ mice exhibit significantly reduced cardiac function (CO: 14.75 µL +/- 0.70 µL) and cardiac hypertrophy (Td: 1.03mm +/- 0.03) compared to WT littermates (CO: 21.44 µL +/- 1.96 µL; Td: 0.92 mm +/- 0.01). RNA-seq and mass spectroscopy analyses revealed a significant upregulation of mRNA expression of hypertrophic markers and increased levels of α-myosin containing the E932Δ variant protein in 9-month-old het *Myh6*$^{E932\Delta}$ hearts compared with WT littermates. Western blot analyses demonstrated no significant differences in the phosphorylation status of cMyBP-C and Phospholamban. However, cardiac Troponin I (cTnI) is significantly hyperphosphorylated. pCa-force analyses showed that skinned myofibrils from 9-month-old het *Myh6*$^{E932\Delta}$ mice exhibit significantly increased maximal tension compared to WT littermates. Cardiomyocytes from 9-month-old Het mice exhibited a significant increase in cell shortening and reduced relaxation velocity.

Conclusions: Our data suggest the *Myh6*$^{E932\Delta}$ variant leads to contractile dysfunction and altered cTnI phosphorylation *in vivo*. By further defining the modulatory role of myosin S2 in cardiac contractility, more specific therapeutic targets can be identified to treat HCM.

Poster #74

Slow MyBP-C in Skeletal Myogenesis: Exploring Novel Functions Beyond the Sarcomere

Akhil Baby[1], James W. McNamara[2], Kalyani Ananthamohan[1], Taejeong Song[1], and Sakthivel Sadayappan[1]

[1]Department of Cellular and Molecular Medicine, The University of Arizona, Tucson - 85721

[2]Murdoch Children's Research Institute, The Royal Children's Hospital, Victoria, Australia - 3052

Background: Slow myosin binding protein-C (sMyBP-C), encoded by the *Mybpc1* gene, is a sarcomeric protein that is expressed in both slow- and fast-twitch skeletal muscles. *Mybpc1* mutations cause distal arthrogryposis (DA) and the neonatal-lethal, lethal congenital contracture type-4 (LCCS-4). Although sMyBP-C is classically known for regulating actomyosin interactions, the mechanisms by which *Mybpc1* mutations lead to severe developmental myopathies remain poorly understood.

Methods and Results: To investigate this, we generated a global sMyBP-C knockout mouse model (C1gKO) using the CRISPR-Cas9 technology. C1gKO pups exhibited whole-body tremors, severe muscle dysfunction, muscle atrophy, dysregulated gene expression, and died within 24 hours of birth. To dissect the underlying molecular mechanism of this phenotype, immortalized primary myoblasts were generated from the wild-type and C1gKO pups and differentiated in vitro using low-serum media. The C1KO myoblasts failed to differentiate, whereas WT myoblasts formed mature myotubes. C1KO myoblasts displayed altered cell-cycle and myosin isoform profiles, and markedly reduced levels of the myogenic regulators MyoD, Myogenin, and Myf5. Notably, adenoviral expression of sMyBP-C alone did not rescue the differentiation defect, suggesting an additional regulatory mechanism. However, adenoviral expression of MyoD and Myf5 induced differentiation in C1KO myoblasts. Therefore, we performed co-immunoprecipitation followed by mass spectrometry to identify sMyBP-C-interacting proteins modulating myogenesis. Among the identified interactors was four-and-a-half LIM domain protein 1 (FHL1), previously reported to interact with the C10 domain of MyBP-C and regulate MyoD expression through RBP-J. FHL1 expression increased during differentiation but was significantly reduced in C1KO myoblasts. Importantly, co-expression of FHL1 and sMyBP-C restored myogenic differentiation in C1KO cells.

Conclusions: Together, these findings uncover a previously unrecognized role for sMyBP-C in regulating skeletal muscle myogenesis through interaction with FHL1, revealing a potential molecular mechanism linking *Mybpc1* mutations to congenital myopathies.

Functional Maturation and Pharmacological Modulation of Cardiac Contractility in High-Throughput Engineered Heart Tissues

Daniel Sauter, Lijo Cherian Ozhathil, Ozan Karaman, Rujing Zhang, Miguel Herrmann, Margarita Bobadilla, Torben Trindkær Nielsen, Roberto Edmundo Ponce Reyes, Tetiana Kapinus, Sandra Wilson
Sophion Bioscience, Ballerup, Greater Copenhagen, Denmark

The myocardium enables powerful contractions essential for circulation and oxygen delivery. Cardiac contractility is a critical parameter for diagnosing cardiac conditions and guiding therapeutic development. Engineered Heart Tissues (EHTs) derived from human induced pluripotent stem cells (iPSCs) have become indispensable for cardiac research, yet most platforms remain low-throughput, costly, and lack reproducibility.

We present a high-throughput 96-well EHT platform featuring a PDMS-free, bioinert consumable manufactured via high-resolution 3D printing, ensuring reproducible pharmacological testing and reduced cell requirements. The EHTs displayed spontaneous contractions for approximately 30 days in culture. Over this period, they responded to electrical pacing and exhibited progressive functional maturation, evidenced by increased contraction amplitude and the development of a positive force–frequency relationship. Electrical pacing enhanced contractile strength while simultaneously reducing the spontaneous beating rate, suggesting adaptive remodeling of the tissue.

Pharmacological profiling confirmed physiological responsiveness: Isoproterenol induced positive inotropic and chronotropic effects; Nifedipine caused negative inotropy.

These results validate the platform’s ability to replicate clinically relevant drug effects and cardiac physiology in vitro, offering a scalable, cost-efficient solution for preclinical cardiotoxicity screening and drug discovery.

Post-Activation Potentiation and the Influence of Estrogen, Age and Regulatory Light Chain

Austin W. Ricci, Rishi Gulati, Julissa Ortiz-Delatorre, Ella Dummer, and Damien M. Callahan
Department of Human Physiology, University of Oregon, Eugene OR.

Pre-clinical evidence supports a direct role of estradiol (E2) in modulating skeletal muscle contractile function via regulatory light chain phosphorylation (RLCp). Indeed, when E2 was experimentally suppressed, acute activation-induced RLCp and associated enhancement in muscle excitability, or post-activation potentiation (PAP), are also impaired. Some clinical evidence supports the notion that RLCp is reduced in postmenopausal females and associated with contractile functional deficits at the cellular level. However, few if any studies have specifically examined the role of E2 in mediating RLCp and response to prior activation in humans. Therefore, the goal of our study was to independently assess the impact of E2 suppression on RLC phosphorylation and PAP in old and younger female participants. We **hypothesized** that younger volunteers with relatively high E2 will exhibit PAP and RLCp that is enhanced when compared with E2 suppressed young and older participants.

METHODS: Three groups of female participants with varying levels of E2 participated: young eumenorrheic (EU; 10), young estrogen suppressed, via progesterone-only hormonal contraceptives (HC; 8) and older, postmenopausal (PM; 5). Participants in Eu group were scheduled during the mid-luteal phase of their menstrual cycle. Blood serum estradiol levels were measured via liquid chromatography-mass spectrometry. Muscle morphology was assessed using ultrasound (Philips Ultrasound Inc., Church Hill, PA) of participants' dominant quadriceps. A Biodex dynamometer (Evome Medical Technologies Inc., Carlsbad, CA) measured voluntary isometric knee extension strength (MVIC) and PAP was assessed via electrical stimulus (1 pulse, 400μs) before and after a 10s maximum knee extension. A muscle biopsy of the vastus lateralis was obtained in rested and post-activation conditions to assess RLCp via isoelectric separation.

RESULTS: There were no main effects of group on muscle size or MVIC**.** Similarly, there were no significant main effects of group on PAP, though Eu tended to have greater PAP for total twitch torque and rate of force development ($p = 0.1$). Further, we found significant, positive correlation between serum E2 and PAP in the pooled group of young participants ($r^2 = 0.47$; $p = 0.028$), and potentiated twitch torque normalized to quadriceps CSA in the total sample ($r^2 = 0.299$; $p = 0.047$). Measures of RLCp are underway and unavailable at the time of abstract submission.

DISCUSSION: Preliminary results are consistent with our hypothesis that E2 provides a protective effect on contractile function at the whole muscle level, especially related to potentiation. Stronger associations between E2 and PAP were observed when older adults were excluded from analyses suggesting aging has independent impact on muscle function that may be independent of E2 and its effect on RLCp. Nevertheless, our data support additional research to better understand the acute and chronic effects of variations in E2 on skeletal muscle in young and older adult females.

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Poster #77

Characterization of a Stress-Induced BAG3 Cleavage Product in Heart Failure

Laura Sherer[1], Ahmed Zied[2], Thomas Martin[2], Michaela Door[1], Jonathan Kirk[1]

1. Department of Medicine, University of Chicago Biological Sciences Division, Chicago, IL
2. Department of Cell and Molecular Physiology, Loyola University Stritch School of Medicine, Maywood, IL

The co-chaperone BAG3 is a major hub of protein homeostasis in the cardiac sarcomere. Overexpression of BAG3 in mice rescues systolic function post-MI, illustrating the cardioprotective role of BAG3 *in vivo*. In humans, mutations in BAG3 can cause dilated cardiomyopathy (DCM). Indeed, multiple BAG3 gene therapy clinical trials are currently underway for patients with BAG3-associated DCM. Despite BAG3's therapeutic relevance, the mechanisms underlying BAG regulation in heart failure are not well-understood—a knowledge gap we sought to address here. We found that BAG3 protein is significantly reduced in human DCM tissue compared to non-failing tissue, whereas the mRNA of *bag3* is increased. This discrepancy suggests that BAG3 protein loss in DCM is post-transcriptionally mediated. To investigate potential post-transcriptional mechanisms, we subjected wildtype mice to an MI and analyzed the tissue four weeks post-surgery. In these samples, we observed a significant post-MI increase in a BAG3 cleavage band at ~74 kDa. A similar cleavage product was also upregulated in human ischemic cardiomyopathy. To characterize this BAG3 cleavage product, we needed to determine the location of the cleavage site. We first identified candidate sites using the ProsperousPlus webtool. We then generated BAG3 variants with alanine substitutions at the prospective cleavage sites and tested them via *in vitro* caspase cleavage assays. Our results showed that the D518A substitution is sufficient for blocking BAG3 cleavage, indicating that cleavage occurs within the intrinsically disordered C-terminal tail of BAG3 at $LEAD^{515\text{-}518}$. Notably, D518A also blocked formation of a secondary cleavage product, suggesting that cleavage after residue 518 promotes further cleavage at subsequent sites. Complementing these *in vitro* cleavage assays, cycloheximide chase experiments in neonatal rat ventricular myocytes also demonstrated that cleaved BAG3 is less stable than its full-length counterpart. Together, these results suggest that cleavage leads to BAG3 instability and may cause BAG3 loss in heart failure, exacerbating cardiac dysfunction. Thus, preventing BAG3 cleavage is a promising therapeutic strategy with the potential to improve patient outcomes. Future work will focus on exploring the functional consequences of BAG3 cleavage in human iPSC-derived cardiomyocytes, engineered heart tissues, and mice.

Spontaneous oscillatory muscle contractions are from sarcomeric origin in novel *MYBPC1* variant associated with myogenic tremor

Ricardo Galli[1,6], Stefan Conijn[1], Daan Hoomoedt[1], Rajvi Balesar[1], Nemo Anno[2], Julia Muldoon[2], Weikang Ma[3-4], Tom Irving[3-4], Josine de Winter[1], Edoardo Malfatti[5], Carsten Bönnemann[6], and Coen Ottenheijm[1-2]

[1]Amsterdam UMC location Vrije Universiteit Amsterdam, Physiology, Boelelaan 1117, Amsterdam, The Netherlands
[2]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, Arizona, USA.
[3]BioCAT, Department of Biology, Illinois Institute of Technology, Chicago, IL, USA.
[4]Center for Synchrotron Radiation Research and Instrumentation, Illinois Institute of Technology, Chicago, IL, USA.
[5]Centre de Référence de Maladies Neuromusculaires, Paris-Est University, France
[6]Current address: National Institutes of Health, National Institutes of Neurological Disorders and Stroke, Maryland, US.

Slow skeletal myosin binding protein C, encoded by MYBPC1, is a sarcomeric protein essential for thick filament regulation. Variants in MYBPC1 have been classically associated with distal arthrogryposis, and more recently with tremor and myopathy. Here, we investigated the impact of a novel homozygous MYBPC1 p.G132R variant on myofiber nanostructure and contractility using permeabilized fibers from patients and a corresponding knock-in mouse model.

Patient-derived myofibers exhibited marked atrophy, reduced maximal absolute and specific force, and unchanged calcium sensitivity. During submaximal activation, these fibers developed spontaneous oscillatory contractions, most prominent at pCa 6.2 (approximately 25–30% of maximal force). Given the role of MYBPC1 in stabilizing the myosin head interacting-head motif, we assessed myosin conformational states using MANT-ATP chase experiments. These revealed a significant shift toward the disordered relaxed (DRX) state in patient myofibers. Consistently, low-angle X-ray diffraction showed an increased I_{11}/I_{10} ratio, increased M3 spacing in both relaxed and activated states, and reduced M3 and M6 intensities, indicating structural disruption of thick filament order.

To further define disease mechanisms, we generated a Mybpc1 p.G132R knock-in mouse model. Ex vivo analysis of the soleus muscle recapitulated the human phenotype, showing reduced absolute force without changes in calcium sensitivity. Additionally, time to peak contraction and half-relaxation time were significantly shortened at maximal and submaximal stimulation frequencies, suggesting increased and less regulated myosin head attachment–detachment kinetics. X-ray diffraction analyses in mouse muscle bundles confirmed increased M3 and M6 spacing in the activated state, decreased intensities in both relaxed and activated states, and an increase in I_{11}/I_{10} ratio during relaxation.

From a therapeutic perspective, treatment with mavacamten, a myosin inhibitor that stabilizes the super-relaxed (SRX) state, significantly reduced both the amplitude and frequency of spontaneous oscillatory contractions in patient myofibers.

Overall, these findings demonstrate that the homozygous MYBPC1 p.G132R variant causes sarcomere-based dysfunction characterized by destabilization of the thick filament OFF state, promoting aberrant myosin head behavior, axial strain, and spontaneous oscillatory contractile activity. Importantly, pharmacological stabilization of myosin suggests a promising strategy to alleviate tremor and muscle dysfunction in affected patients.

Cryo-EM Reveals How Cardiac Drugs (Mavacamten, Omecamtiv Mecarbil), and Cardiomyopathy Mutation (E525K) Modulate the Myosin Interacting-Heads Motif

Arun Kumar Somavarapu[1], Jinghua Ge[2], Ruchi Gautam[1] Christopher M. Yengo[2], Roger Craig[1], and Raul Padron[1]

[1]Division of Cell Biology and Imaging, Department of Radiology, University of Massachusetts Chan Medical School; Worcester, MA, USA

[2]Department of Cell and Biological Systems, Pennsylvania State University, College of Medicine; Hershey, PA, USA

Genetic mutations in myosin, the motor protein that powers the heartbeat, are linked to inherited hypertrophic (HCM) and dilated (DCM) cardiomyopathies. Mavacamten (Mava) and omecamtiv mecarbil (OM) are therapeutic, myosin-targeted drugs designed to treat these myopathies, but their mechanism of action has remained unclear. Using single-particle cryo-EM, we determined near-atomic resolution structures of wild-type (WT), mavacamten-bound, and omecamtiv mecarbil-bound cardiac myosin constructs in the interacting heads motif (IHM) state. Across all conditions, we observed two distinct, previously unreported conformations of the IHM, in which the S2 tail was bent and docked with the free head (S2-FH docked) or straight and detached from the free head (S2-FH undocked). We show how Mava stabilizes the S2-FH docked conformation by reinforcing key electrostatic interfaces in the molecule, whereas OM weakens these interfaces, favoring the S2-FH undocked structure, leading to open, non-interacting heads state. This drug-induced remodeling elucidates previously unclear allosteric mechanisms through which these drugs either inhibit or enhance myosin activity, countering the deleterious impacts of disease.

We further examined the DCM-associated E525K mutation in the myosin head to investigate its structural mechanism. While WT myosin exists in both the docked and undocked states, the E525K mutant adopts only the docked structure, with reduced conformational variability, indicating a shift in the conformational equilibrium. The E525K mutation selectively restricts S2 mobility and enhances inter-domain contacts, stabilizing the S2-FH docked IHM structure by reinforcing the electrostatic interactions at S2-BH interface. Together, these findings demonstrate that both the small-molecule drugs (Mava and OM) and disease-associated E525K mutation modulate myosin structure through distinct mechanisms that act on a common electrostatic interaction network to control cardiac contractility.

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Characterizing Skeletal Muscle Dysfunction in Hereditary Myopathy with Early Respiratory Failure (HMERF): *Insights from Human Muscle Biopsies*

Seong-won Han[1], Jennifer Roggenbuck[2], Johan Lindqvist[1], Zaynab Hourani[1], Jochen Gohlke[1], Marco Tellez[2], John Smith[1], Justin Kolb[1], Henk Granzier[1]

[1]Cellular and Molecular Medicine, University of Arizona
[2]Wexner Medical Center, The Ohio State University

Introduction

Hereditary Myopathy with Early Respiratory Failure (HMERF), caused by mutations in A-band titin, is characterized by progressive proximal and distal skeletal muscle weakness and respiratory failure. Due to its recent discovery, the intrinsic muscle properties of affected patients remain poorly understood. Here, we examined muscle properties in HMERF patients across biological scales.

Participants and Methods

Five HMERF patients (41-59 years old) from 4 families were included. The patients exhibited progressive skeletal muscle weakness with respiratory involvement, although disease severity varied. Tibialis anterior biopsies were obtained from patients and healthy controls. For single-fiber mechanics, samples were stored overnight in skinning solution (4°C), then rinsed and maintained in relaxing solution for experiments (no freezing or glycerol storage). After experiments, fibers were stored at −80°C for fiber type identification. Samples were also stored at -80°C for protein analysis, or maintained in RNA *later* solution for RNA sequencing.

Results

Single-fiber mechanics were assessed in controls (N=5, 75 fibers) and HMERF patients (N=5, 71 fibers). No difference was observed in passive force across sarcomere lengths ranging from 2.0 to 3.5 µm. In contrast, active mechanical properties were impaired in HMERF fibers. Specifically, single fibers from HMERF patients exhibited reductions in maximum active force (−34%, p=0.056), active stiffness (−51%, p=0.018), and rate of force redevelopment (−34.3%, p=0.025) compared to controls, while calcium sensitivity remained unchanged. Type I fibers were more affected, showing greater deficits in maximum active force and stiffness compared to Type II fibers. Protein quantification revealed no difference in fiber type composition in the biopsies, and total titin and myosin heavy chain content were also unchanged in HMERF muscle relative to controls.

Conclusions

The absence of changes in passive force and titin content indicates that HMERF mutations have minimal impact on titin-based passive stiffness at the sarcomere level. In contrast, muscle fibers with HMERF mutations exhibited significant reductions in active force, suggesting that the underlying mechanism may involve a reduction in myofibrillar fractional area and/or impaired cross-bridge kinetics. The impact of HMERF mutations is more severe on Type I fibers, the molecular basis of which warrants further study.

THE D73N TROPONIN C MOUSE: A TRANSLATIONAL MODEL OF DILATED CARDIOMYOPATHY

Ali Ulker, Amelia Van Aelst, Jordan King, Shadie Mirib, Radmila Terentyeva, Fruzsina Perger, Anastasia Pokrass, Nipun Malhotra, Svetlana Tikunova, Mona El Refaey, Peter Reiser, Sandor Gyorke, Dmitry A Terentyev, Jonathan P Davis

Dilated cardiomyopathy (DCM) is a major cause of heart failure, defined by ventricular dilation and reduced systolic performance. To dissect the molecular underpinnings of DCM, we employed a knock-in mouse model expressing the D73N mutation in cardiac troponin C (cTnC). This targeted mutation, introduced into the Ca^{2+}-binding regulatory N-domain of cTnC, accelerates Ca^{2+} dissociation, thereby desensitizing myofilaments to Ca^{2+}. These mice recapitulate key features of human DCM, including reduced ejection fraction, ventricular dilation, and early mortality.

Notably, these mice also exhibit elevated mitochondrial reactive oxygen species (ROS) levels, consistent with increased oxidative stress in failing myocardium. Furthermore, a subset of D73N mice develop left atrial thrombi, pathologically relevant finding that may reflect altered biomechanical signaling within the stressed cardiac microenvironment.

Despite these severe cardiac changes, no functional deficits were observed in peripheral tissues. Cage-side observations, exercise tolerance, diaphragm contractility, carotid artery structure, and skeletal muscle force output were all preserved compared to wild-type controls. Further, whole-body metabolic parameters, glucose tolerance, and insulin sensitivity also remained unchanged, confirming that the D73N model is cardiac-specific and not confounded by systemic dysfunction.

Together, these findings establish the D73N knock-in mouse as a highly specific and translationally relevant model of DCM, distinguished by cardiac-restricted dysfunction, oxidative stress, and the emergence of atrial thrombi. This model is ideal for testing therapies aimed at improving contractility, reducing oxidative stress, or preventing maladaptive cardiac remodeling.

Frailty-on-a-Chip: Developing a Microphysiological Model of Age-Related Skeletal Muscle Dysfunction

Anicca D. Harriot[1,2], Zhanping Ren[2,3], Buel Rogers[4], and Deok-Ho Kim[2,3,5,6]

1 Division of Geriatrics and Gerontology, Johns Hopkins University, Baltimore, MD
2 Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD
3 Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD
4 AAVogen, Baltimore, MD 5)Department of Medicine, Johns Hopkins University, Baltimore, MD
5 Institute for NanoBio Technology, Johns Hopkins University, Baltimore, MD
6 Center for Microphysiological Systems, Johns Hopkins University, Baltimore, MD

To investigate age-related dysfunction in a human relevant model, we leveraged our recently established human microphysiological system (hMPS) whereby engineered muscle tissues (EMTs) are generated from myoblasts from young adult (age 20-25) and older adult (> 65 years) donors. Our muscle hMPS is an integrated platform that allows for real-time, longitudinal measurement of force production, contractile kinetics, and Ca^{2+} signaling simultaneously across a 24-well plate system. We fabricated EMTs and profiled these outcomes in (1) a case study comparing young vs older adult donors and (2) a second case study across a spectrum of inclusion body myositis donors ranging from healthy to severe. Here we find no significant reduction in contractile function in EMTs derived from older adults compared to young. However, when stratified by donor, our results indicate disparate functional outcomes in the aging population – reflective of heterogeneity of aging phenotypes in older adults. In IBM patients, we were able to recapitulate the clinical stratification of disease phenotype in our muscle-on-a-chip hMPS. This work represents a comprehensive, systematic and high-throughput approach to develop an *in-vitro* model of age-related muscle dysfunction. Our skeletal muscle hMPS supports rapid screening of a diverse population of donors with a larger number of biological replicates simultaneously to improve reproducibility. Moreover, high-throughput, high-content analyses of contractile kinetics and Ca^{2+} transients have allowed our lab to investigate multiple conditions and interventions in a single experiment, accelerating discovery and increasing efficiency all while reducing costs.

Myosin Mutations and Purine Metabolism Impact Contractile Function, Cytoskeletal Structure, and Mitochondrial Function of Human iPSC-derived Cardiomyocytes

Authors: Alison Vander Roest[1], Nathan French[1], Seungyeon Julia Han[1], Dan Beard[2]

Affiliation: [1] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA
[2] Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA

Understanding the molecular and cellular scale processes influencing force generation, mechanosensing, and structural remodeling in the context of hypertrophic cardiomyopathy (HCM) has been a major focus of my lab. Tissues from patients with HCM and heart failure show depleted metabolic capacity as well as changes in intracellular and extracellular protein structures that can further alter the efficiency of contraction, but the mechanisms and interactions of these phenotypes is incompletely understood. We use genetic and pharmacologic perturbation of Human induced pluripotent stem cell (hiPSC) derived cardiomyocytes (CMs) in engineered environments to assess the functional impact of altered contractile force, energetic supply and remodeling. We micropattern polyacrylamide gels to replicate the mechanical stiffness and cell alignment of native and diseased tissues and measure single cell forces with traction force microscopy simultaneously with myofibril remodeling and sarcomere contraction before and after the addition of an ADSS inhibitor to prevent normal maintenance of adenonucleotides. In this platform we also measured the ATP:ADP ratio control and mutant hiPSC-CMs using the Perceval HR reporter after pharmacologic inhibition of normal ATP/ADP nucleotide cycling. We have confirmed an expected increase in force production by single micropatterned hiPSC-CMs with the H251N HCM myosin mutation and a surprising increase in the force production of cells with a homozygous DCM associated R369Q mutation and sarcomere shortening and myofibril remodeling in response to purine metabolism modulation. We also observed decreased ATP:ADP ratio in the HCM hiPSC-CMs and decreased force and mitochondrial respiration in both control and HCM cells after pharmacologic nucleotide depletion, highlighting that increase ATP demand can disrupt normal ATP maintenance, and that depleted ATP supply impairs mitochondrial and mechanical function. Our ongoing and future work focuses on mechanisms by which cell phenotype shifts contribute to altered function in disease.

Poster #84

Assessing two novel nemaline myopathy mouse models for future therapeutic studies targeting myosin dysfunction

Fanny Rostedt[1,2], Sonja Heininen[1,2], Justin Kolb[3], Henk Granzier[3], Jenni Laitila[1,2]

[1]Folkhälsan Research Center, Helsinki, Finland

[2]Department of Medical Genetics, University of Helsinki, Helsinki, Finland

[3]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA

Nemaline myopathy-causing mutations in nebulin (*NEB*) disrupt the thin filament structure and actin-myosin interactions. In relaxed muscle, myosin heads can be found in two distinct conformations, the energy-conserving super-relaxed state (SRX) and the energy-consuming disordered-relaxed state (DRX). The DRX/SRX ratio has previously been found to be disrupted in nemaline myopathy (NM) muscle.

The aim of this study was to compare two novel NM mouse models: the humanized *Neb*-del ex55 and the compound heterozygous *Neb*-del ex55/missense ex106 model and to evaluate their suitability for future therapeutic trials with myosin-modulating drugs. Using the Mant-ATP chase method, we determined the myosin DRX/SRX ratio in relaxed single muscle fibres. Cross-sectional analysis (CSA) of the soleus muscle was performed to assess fibre-type distribution and fibre size.

The Mant-ATP chase experiments demonstrated that the humanized *Neb*-del ex55 model consistently exhibited a high proportion of DRX myosins, whereas the compound heterozygous *Neb*-del ex55/missense ex106 model showed greater variability. Both models exhibited a predominance of type I fibres and small type IIA fibres. Based on the Mant-ATP chase experiments and CSA measurements, the humanized *Neb*-del ex55 model presented with a more homogenous phenotype and could therefore be better suited for future studies involving myosin modulating drugs, such as mavacamten or aficamten.

Small Molecule Z06 Targets Tropomyosin Overlap Flexibility and Improves Systolic Function in a Dilated Cardiomyopathy Mouse Model

Catherine Vasquez[1]; Melissa Lynn[1]; Romi Castillo[1]; Andrew Kim[1]; Jil C. Tardiff[1]

[1]University of Arizona, Tucson, Arizona, United States

An important component of cardiac myofilament activation is the overlap structure between neighboring tropomyosin (Tm) molecules and the N-terminal extension of cardiac troponin T. Previous studies have shown that the DCM-linked tropomyosin mutation, D230N-Tm, decreases the distance across the center of the overlap resulting in a compaction in this region and a concomitant decrease in flexibility. Given the mutation-induced structural changes, we screened for molecules that could target this compaction utilizing a fully atomistic cardiac thin filament model. Z06 was identified and shown to alleviate compaction of the overlap in D230N-Tm in both our model and in fully reconstituted cardiac thin filaments. We hypothesized that directly targeting the structural changes at the overlap can improve regulatory and cardiac function in D230N-Tm mice. To first investigate if Z06 could improve myofilament activation we performed an NADH-coupled ATPase assay at varying calcium concentrations. D230N-Tm myofibrils demonstrated a reduced Hill slope (0.79±0.19) compared to its Non-transgenic (NTg) littermates (1.70±0.35) supporting a decrease in cooperativity of myofilament activation. In the presence of 10um Z06 the Hill slope of D230N-Tm increased (1.27±0.14) and was restored to NTg levels. Similarly, the addition of Z06 improved contractility parameters in isolated cardiomyocytes. D230N-Tm cells exhibited a delayed time to peak, time to peak 50% and time to baseline 50%, and the addition of 10uM Z06 was able to restore these parameters to NTg levels. Finally, we performed preclinical studies in our transgenic mice to investigate any improvement in systolic function with Z06 administration. We administered Z06 to 2-month-old D230N-Tm mice and their NTg littermates. At baseline, D230N-Tm mice exhibited a decrease in percent fractional shortening (%FS) of 18.84±0.405 compared to their NTg littermates (28.59±0.67). After four weeks of treatment we observed an improvement in %FS in D230N-Tm mice to 25.19±0.8127 with no significant changes to systolic function in NTg littermates. Future work will investigate the efficacy of Z06 as a potential late-stage treatment and assess its general applicability in other thin filament mutations, such as, D219N-Tm (HCM).

Poster #86

Pulsatile Myofilament Activity in Myotrem Myopathy Associated with Myogenic Tremor

Jennifer M.C. Mariano[1]*, Laurin M. Hanft[2]*, Suhan Cho[1], Christopher W. Ward[3], Kerry S. McDonald[2], and Aikaterini Kontrogianni-Konstantopoulos[1]

*These authors contributed equally to this work

[1]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201. [2]Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, MO 65212. [3]Department of Orthopedics, University of Maryland School of Medicine, Baltimore, MD 21201.

Myosin-binding protein C (MyBP-C) comprises a family of myofilament proteins that maintain sarcomeric structure and regulate actomyosin crossbridge cycling. Pathogenic variants in *MYBPC1*, the gene encoding the slow skeletal isoform (sMyBP-C), lead to a dominant congenital myopathy, termed Myotrem, characterized by muscle weakness, hypotonia, and a distinctive tremor of myogenic origin, in the absence of neuropathy. However, the molecular mechanism(s) of myogenic tremorgenesis is largely unknown. One potential mechanism is aberrant myofilament stretch activation, which is defined as a delayed increase in force after a rapid stretch. We utilized the Myotrem murine model harboring the pathogenic *MYBPC1* E248K variant to test the hypothesis that stretch activation is augmented in permeabilized Myotrem E248K soleus fibers. We found that stretch activation was significantly increased in E248K soleus muscle fibers. Interestingly, once submaximally Ca^{2+}-activated, a subpopulation of slow-twitch E248K fibers exhibited spontaneous pulsatile sarcomere oscillations. This pulsing behavior generated a sinusoidal waveform pattern in sarcomere length, which often persisted on a timescale of minutes. These results align with sMyBP-C as key regulator of the synchronous activation of myofilaments by dampening both spontaneous oscillatory activity and stretch-dependent activation. We propose that the presence of sMyBP-C-E248K disrupts this regulation, thereby driving pathogenic myogenic tremors.

Understanding the Mechano-Signaling Role of the Z-disc in the Pathogenesis of Hypertrophic Cardiomyopathy

Eslem N. Yürümez[1], Daan Hoomoedt[1], Nicole N. van der Wel[2], Connie R. Jimenez[3], Sanne A. Kleijn[1], Flynn J. de Heer[1], Jolanda van der Velden[1], Diederik W.D. Kuster[1]

[1]Department of Physiology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Cardiovascular Sciences, The Netherlands; [2]Department of Medical Biology, Electron Microscopy Centre, Amsterdam UMC, Amsterdam, The Netherlands; [3]Department of Medical Oncology, VUmc Cancer Center Amsterdam, OncoProteomics Laboratory (T.V.P., C.R.J.), Amsterdam UMC, The Netherlands

Introduction

Hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disorder, affects 1:500 people. Characterized by left ventricular myocardium thickening, it is associated with hypercontractility and impaired relaxation, that precedes hypertrophy. Mutations in sarcomeric proteins, particularly *MYBPC3*, are the most prevalent cause but the pathogenesis remains unclear. The Z-disc, connecting sarcomeres, plays a pivotal role in mechano-signaling by integrating both mechanical stress from contracting sarcomeres and external forces through cytoskeletal coupling. However, its role in HCM is poorly understood.

Aim

Our aim is to define the role of the Z-disc, connecting it to intracellular and extracellular stress and how its remodeling contributes to HCM pathogenesis.

Materials and Methods

Z-disc thickness and morphology analyzed in electron microscopy (EM) images from HCM patients' myectomy tissue (n=53) and non-failing donor samples (n=23). Proteomic and phosphoproteomic analyses were performed septal tissue of HCM patients (n=24) and donors (n=8). Kinases that phosphorylate Z-disc proteins were identified using kinase-recognition motif analysis. A *MYBPC3* c.2373insG hiPSC-CM cell line was used to investigate mutation effects on Z-disc structure and function *in vitro* under contractile stress with myosin inhibitors and activators.

Results

EM analysis revealed increased Z-disc thickness and altered morphology in HCM samples. Proteomic analysis identified higher expression of Z-disc proteins but not sarcomere proteins in HCM with differential phosphorylation. Kinase analysis predicted reduced PKA and increased p38 activity as important regulators of Z-disc phosphorylation. Preliminary 2D and 3D analysis of the *MYBPC3* cell line indicated Z-disc changes under increased contractile stress.

Conclusions

HCM disrupts Z-disc function and composition, linking sarcomeric mutations to altered mechano-signaling. Identified signaling pathways and Z-disc proteins offer potential therapeutic targets for HCM treatment.

Alternative Splicing of *MYBPC1* in Healthy and Diseased Skeletal Muscle

Brenna McIntyre[1], Kalyani Ananthamohan[1], Paulo Pires[1], Taejeong Song[1], and Sakthivel Sadayappan[1]

[1]Department of Cellular and Molecular Medicine, University of Arizona College of Medicine, Tucson, Arizona, USA.

Background: *MYBPC1*, encoding slow-twitch myosin-binding protein-C (sMyBP-C), is a sarcomeric protein important for the regulation of myosin cross-bridge cycling in slow-twitch skeletal muscle myofibers. *MYBPC1* undergoes alternative splicing, primarily at exons encoding the NH2 and COOH termini, producing more splice variants than its other two paralogs combined. These splice variants are well conserved within mammals, and many remain uncharacterized and understudied. Three of the C-terminal alternatively spliced variants occur at exons that encode the very distal end of the final functional domain of the sMyBP-C protein, predicting structural changes that may interfere with protein localization and function.

Methods: We developed a real-time polymerase chain reaction (RT-PCR) assay that accurately measures the expression of these C-terminal transcriptional variants in *mus musculus* to characterize their relative expression in aging and disease. We excised slow-twitch and fast-twitch muscles from wildtype (WT) and Duchenne Muscular Dystrophy (DMD) mouse models and isolated mRNA to measure C-terminal variant *Mybpc1* expression at five different aging timepoints and in myopathic disease progression using our assay.

Results: Long-RNA sequencing of mouse slow- and fast-twitch muscles identified three splice variants of *Mybpc1* located within the last two protein-coding exons of the transcript. Utilizing our variant-specific RT-PCR assay, we found that expression of each variant changes throughout aging in both a tissue-type-specific and sex-specific manner. While the overall expression of each variant changes throughout aging, we found that the relative expression ratio of each variant within each time point remains the same. In DMD muscles, we found that C-terminal variant expression was significantly altered compared to aged-matched WT. This effect was limited to slow-twitch muscle, with no significant changes in myofiber type composition at the transcriptional level.

Conclusions: Our study found that the expression of three alternatively spliced C-terminal variants of *Mybpc1* are altered during aging and during myopathic disease progression. These data suggest alternative splicing of *Mybpc1* plays a role in the muscle aging process and in the disease response, providing insight into the potential factors driving *Mybpc1* alternative splicing to possibly identify a therapeutic target for skeletal muscle diseases.

Cardiac Hypercontractility as a Causal Mechanism of Congenital Heart Disease

Michele Zanetti[1], Anea Whitfield[2], Xia Li[1], Martina Brueckner[2], and Stuart G. Campbell[1]

[1]*Department of Biomedical Engineering, Yale University, New Haven, Connecticut, United States*
[2]*Department of Pediatrics, Yale School of Medicine, New Haven, Connecticut, United States*

Variants of cardiac myosin-binding protein C (*MYBPC3*) are well-known causes of adult-onset hypertrophic cardiomyopathy (HCM). Recently, *MYBPC3* variants have also been identified in pediatric patients with congenital heart disease (CHD). Their impact on the developing heart, however, remains unclear.

Here, we characterized the contractile phenotype of an *MYBPC3* mutation ($MYBPC3^{F1183L/F1183L}$) identified in a patient with CHD presenting with double-outlet right ventricle and coarctation of the aorta. We have introduced $MYBPC3^{F1183L/F1183L}$ into a wild-type hiPSC line and differentiated cardiomyocytes and engineered heart tissues (EHTs) for in vitro functional assays.
We found that $MYBPC3^{F1183L/F1183L}$ results in a drastic *MYBPC3* protein deficiency and a hypercontractile phenotype characterized by increased EHT force production, faster relaxation time, increased diastolic stiffness, and sarcomere disarray compared to WT.
Monoallelic $MYBPC3^{WT/F1183L}$ EHTs showed no evidence of protein haploinsufficiency and were mildly hypercontractile, with no other detectable impairments, either functionally or structurally. This very mild phenotype of $MYBPC3^{WT/F1183L}$ EHTs is consistent with the absence of cardiac abnormalities observed in the heterozygous parents.

To investigate the relationship between *MYBPC3* loss and embryonic structural heart development, we knocked down *MYBPC3* using both morpholino constructs and CRISPR in *Xenopus Tropicalis* tadpoles. *X. Tropicalis* hearts showed an underdeveloped ventricle, valves, and outflow tract, mirroring the aortic arch hypoplasia, coarctation of the aorta, and an underdeveloped left ventricle observed in the $MYBPC3^{F1183L/F1183L}$ patient. Finally, to further elucidate the effect of hypercontractility on heart development, we treated WT tadpoles with the small molecule Omecamtiv mecarbil (OM), a selective myosin activator that increases contraction, thereby reproducing the hypercontractility observed in $MYBPC3^{F1183L/F1183L}$ EHTs. OM-treated tadpoles showed structural abnormalities similar to those observed with *MYBPC3* knockdown.

These results reproduce the patient phenotype, consisting of left ventricular outflow tract obstruction and coarctation of the aorta, establishing a causal link between hypercontractility and structural CHD, and supporting the hypothesis that cardiomyocyte hypercontractility during early cardiogenesis can drive the development of structural heart defects.

Mechanical Ventilation-Induced Diaphragm Weakness and Thick Filament Structural Alterations Persist Following Spontaneous Breathing

Marloes Van Den Berg[1], Wout Claassen[1], Fleur van Brakel[2], Nimo Annor[1], Stefan Conijn[1,2], Julia Muldoon[1], Olga Alekhina[1], Ricardo Galli[1,3], Henk Granzier[1], Weikang Ma[4], Tom Irving[4], Coen Ottenheijm[1,2].

[1] Department of Cellular and Molecular Medicine, Molecular Cardiovascular Research Program, University of Arizona, Tucson, AZ, USA.
[2] Department of Physiology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Center, Amsterdam, Netherlands.
[3] National Institute of Neurological Disorders, National Institutes of Health, Bethesda, MD, USA.
[4] BioCAT, Illinois Institute of Technology, Lemont, IL, USA.

Background: Mechanical ventilation (MV) is lifesaving but frequently causes diaphragm weakness and weaning difficulties, increasing morbidity and mortality in the ICU. The underlying mechanisms include myofilament-based contractile dysfunction, though whether these resolve after weaning remains unclear.

Methods: Male Sprague Dawley rats underwent no MV (NMV), 18h MV, or 18h MV with 24h spontaneous breathing recovery (n=8/group). Diaphragm strips were used for ex vivo contractility studies; remaining tissue underwent small-angle X-ray diffraction, MANT-ATP chase, and myosin regulatory light chain (RLC) phosphorylation assays.

Results: Maximum diaphragm tension was reduced by ~26% after 18h MV (197±29 vs 145±20 mN/mm², p<0.0001) and did not fully restore after 24h recovery (162±16 mN/mm², p=0.005 vs NMV). Optimal length for contraction was reduced after MV (22.8±2.0 vs 20.8±1.4 mm, p=0.02) and partially recovered. X-ray diffraction revealed reduced M6 spacing after MV at rest (7.17±0.01 vs 7.16±0.01 nm, p=0.02) and during activation (7.20±0.02 vs 7.18±0.01 nm, p=0.04), indicating reduced axial strain on the myosin filament, which restored after 24h recovery. Resting M3 intensity, a measure of myosin head ordering, was specifically reduced in the recovery group compared to controls (14.84±4.15 vs 9.96±2.29 a.u., p=0.034), while no difference was observed after 18h MV alone. Upon calcium activation, M3 intensity decreased as expected in NMV (p=0.05) but showed no change after 18h MV (p=0.78) and paradoxically increased after recovery (p=0.05), suggesting altered myosin head organization after MV that does not resolve during recovery. These structural changes were not accompanied by alterations in the equatorial reflections, nor in biochemically measured SRX/DRX ratios, despite significant RLC dephosphorylation after 18h MV that did not fully restore during recovery.

Conclusions: 18 hours of MV causes significant diaphragm weakness that does not fully resolve after 24 hours of spontaneous breathing. Structural thick filament remodeling, including altered myosin head organization and RLC dephosphorylation, persists alongside sustained weakness. These findings suggest that the diaphragm remains structurally vulnerable during early post-weaning recovery, potentially increasing the risk for weaning failure or respiratory complications.

Developing single cell perfusion and contractility setup for cardiomyocytes to understand cell-to-cell variability in drug response

Ilse S. Altenburg[1], Daan Hoomoedt[1], Valentijn Jansen[1], Michiel Helmes[2], Niek Welkenhuysen[3], Gavin Jeffries, Sander Piersma[4], Connie Jimenez[4], Jolanda van der Velden[1], Diederik W.D. Kuster[1]

To understand disease mechanisms or test new drugs in hit-to-lead development, the contractile effects of compounds are often assessed in isolated adult murine cardiomyocytes. Unloaded shortening is measured in dishes with or without compound addition. Because of high variability between cells and the difficulty of paired measurements, a large number (60-100 cells/condition) are required for reproducible results. This bulk method does not allow studying compound kinetics, individual cell responses or combine contractile measurements with molecular analysis in single cells.

Our aim is to measure the molecular mechanism behind cell-to-cell variability in contractile response to drugs. We have developed a method that combines single cell perfusion with contractile measurements by integrating and optimizing the controlled flow capabilities of the Biozone6 with the large scale contractility measurements of the MultiCell. We defined a perfusion are suitable for a single cardiomyocyte and evaluated our method by testing the response of individual cardiomyocytes to the beta-adrenergic receptor agonist isoproterenol (iso). Isolated mouse and rat cardiomyocytes were perfused for 30 seconds with Tyrode's solution, before switching to iso (15nM) for 120 seconds. Contraction increased within 5 seconds after solution switching to iso, reaching a plateau after 40 seconds (n≈57 cardiomyocytes per condition, N=5 mice). Large variation in individual response of cardiomyocytes was observed, with a ~25% of cells showing minimal response to iso. The perfusion system can switch among six different solutions, allowing individual dose response testing (1, 5 and 15nM iso (n=55 N=4)), showing that only a small percentage of cells respond to 1nM. By perfusing individual cells with lysis buffer followed by proteomic analysis, we are assessing the minimum number of cardiomyocytes required to perform proteomics. This will be used to understand the molecular mechanism of drug response (responders vs non-responders),

In conclusion, we are able to assess single cell contractile response to compounds in a dose dependent manner, assess kinetics of drug effects and soon will be able combine molecular and contractile analysis in individual cells. Next, we will focus on if cell-to-cell variability in drug-response increases in cardiac disease.

[1] Dept. of Physiology, Amsterdam University Medical Centers, Amsterdam
[2] CytoCypher BV, Amsterdam, The Netherlands
[3] Fluicell AB, Mölndal, Sweden
[4] OncoProteomics Labaratory, The Netherlands

Poster #92

Myotube models for studies on C-terminal titin variants

Jaakko Sarparanta, Per Harald Jonson, Helena Luque, Emma James, Charlotte Walder, Marco Savarese & Bjarne Udd

Myofin, Folkhälsan Research Center, Helsinki, Finland

Variants affecting titin's C-terminal domain M10 cause C-terminal titinopathies—tibial muscular dystrophy (TMD), juvenile-onset recessive titinopathy, and limb-girdle muscular dystrophy type R10. Some variants, including the Finnish TMD mutation FINmaj, cause a proteolytic loss of a larger part from titin C-terminus. We believe that this "pathological cleavage" is a key event in the pathomechanism of TMD and a possible therapeutic target. Cleavage propensity could also explain the different modes of inheritance and clinical pictures of the variants.

Functional studies on titin are limited by the huge size of the protein. To overcome this challenge, we developed the C2C12-CTE (C-terminal titin exchange) system where heterospecific lox sites were introduced flanking the last *Ttn* exons in C2C12 myoblasts. Transfection of these recipient cells with a donor plasmid and Cre mRNA results in replacement of the targeted *Ttn* region with a gene cassette from the donor construct, theoretically allowing quick and economical introduction of variants, tags or other modifications to C-terminal titin. While we have successfully used the system to establish recombinant cells, some practical challenges remain. Our original intention was to use the recombinant cells as a polyclonal pool, but non-specific donor integration necessitates cloning of recombinants, leading to lower throughput and possible clonal differences. We are attempting to increase the recombination frequency by optimizing the transfection and through chemical cell cycle arrest, and planning to introduce a negatively selectable fluorescent marker allowing FACS isolation of recombinant cells.

Our first application for the C2C12-CTE system is studying the pathological cleavage of titin C-terminus in live cells. For this, we established cell clones with wild-type or FINmaj titin fused to fluorescent protein tags (eGFP or mScarlet3). In wt-GFP/FINmaj-mScarlet3 hybrid myotubes, FINmaj titin showed an apparently normal M-band signal, suggesting that the pathological cleavage unexpectedly does not occur in 2D-cultured myotubes. We are currently producing cells with a V5 tag to investigate the possibility that the folded fluorescent protein protects titin from cleavage. Longer-term plans include introducing an additional fluorescent marker to the donor cassette for confirming the incorporation of mutant myoblasts into the myotubes, and testing the cleavage in a 3D setup.

Poster #93

Molecular basis of length-dependent activation in cardiac muscle.

Alice Arcidiacono[1], Yanhong Wang[1], Emma Smith[1], Michaeljohn Kalakoutis[1], Cameron Hill[1], Theyencheri Narayanan[2], Malcolm Irving[1], Luca Fusi[3] and **Elisabetta Brunello**[1].

[1]Randall Centre for Cell and Molecular Biophysics and British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom.

[2]European Synchrotron Radiation Facility, Grenoble, France

[3]Centre for Human and Applied Physiological Sciences, King's College London, London, United Kingdom.

Length-Dependent Activation (LDA), the increase in calcium sensitivity of force at longer sarcomere length, is considered the cellular equivalent of the Frank-Starling law of the heart, but its underlying molecular mechanisms remain unclear. Here we investigated the role of thin- and thick-filament regulation in LDA in demembranated trabeculae isolated from rat hearts. We used fluorescence polarisation from exogenous bifunctional rhodamine probes either in the N- or C-terminal lobes of troponin-C (TnC), and in the regulatory light chain (RLC) of myosin to characterise the sarcomere length dependence of the structural changes in thin and thick filaments, respectively. Trabeculae were activated at steady-state calcium concentration under sarcomere length-isometric conditions by temperature jump to 27°C, in the presence of 3% Dextran T-500, at short (2.1 μm) and long (2.3 μm) sarcomere length. The increase in sarcomere length increased the calcium sensitivity of both force and the orientation changes in the RLC probe by 0.2 pCa units. In contrast, the calcium sensitivity of the thin filament measured by the TnC probes was not altered by the increase in sarcomere length. Addition of the myosin inhibitor Mavacamten (25 μM) completely inhibited active force at both sarcomere lengths and locked the myosin motors in the OFF state on the thick filament independently of calcium concentration and sarcomere length. Mavacamten also significantly desensitised the thin filament to calcium, independently of sarcomere length. We further characterised the effect of sarcomere length on the regulatory state of the thick filament using Small-Angle X-ray Diffraction on demembranated trabeculae. We showed that a rapid (5ms) increase in sarcomere length from 2.0 μm to 2.3 μm triggered the activation of the myosin motors on the thick filament in demembranated trabeculae activated at submaximal calcium concentration (pCa 6), but not in the absence of calcium (pCa 9). All together our results indicate that cardiac LDA is mediated by length-dependent regulatory structural changes in the thick filament which require thin filament activation.

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Characterization of Skeletal MyBP-C Expressions in Health and Diseased Muscles

Jessica Spring, Akhil Baby and Taejong Song

Department of Cellular and Molecular Medicine, University of Arizona College of Medicine, Tucson, Arizona, USA

MyBP-C is a key sarcomeric regulatory protein that has three paralogs: slow (sMyBP-C), fast (fMyBP-C) and cardiac (cMyBP-C). Of these, only sMyBP-C and fMyBP-C are expressed in skeletal muscle, and mutations in these proteins have been correlated with diseases like distal arthrogryposis, myogenic tremor and lethal congenital contracture syndrome. Previous research done in our lab showed they play an important role in generation of maximum speed and force contraction, sarcomeric integrity, calcium sensitivity, muscle growth and development. However, the expression profiles of sMyBP-C and fMyBP-C in both healthy and diseased states remain unclear. In particular, their localization, post-translational modifications, and distribution of splicing variants in human skeletal muscle have not yet been clearly characterized.

Previous experiments were done in our lab to assess the role of fMyBP-C in health and disease using three types of mice: diabetic, Duchenne muscular dystrophy, and wild type. Western blot analyses of tibialis anterior muscle showed a significant reduction in fMyBP-C in diabetic mice compared to wild type. On the other hand, sMyBP-C levels were significantly higher in both Duchenne and diabetic mice. Additionally, eccentric contraction-induced muscle injury in gastrocnemius muscle resulted in a progressive decline in both fMyBP-C and sMyBP-C expression levels. These findings suggest that loss of these proteins from injury and disease may contribute to functional alterations in skeletal muscle. Other experiments using plantaris muscle to evaluate sex-based differences in muscle composition were done. We observed significantly higher fMyBP-C expression in males whereas females exhibited increased sMyBP-C phosphorylation levels. These results show that there are sex-dependent differences in the composition and regulation of skeletal muscle.

To expand these findings to humans, we decided to profile human skeletal muscle samples. We obtained 9 human skeletal muscle samples (5 female, 4 male) from the NIH which were fixed in formalin and stored in a 70% ethanol solution. These samples came from patients with varying ages, BMIs and health statuses. Experiments for these samples, including western blotting, RNA-seq and immunohistochemistry, are still ongoing and will help us better understand the role of MyBP-C in human skeletal muscle in health and disease.

Modeling the Impact of Preterm Birth on Myocyte Crossbridge Kinetics and Force

Salla M Kim[1], Daniel A Beard[2], Anthony J. Baker[3], Kara N Goss[4], Mitchel J Colebank[5], and Naomi C Chesler[1]

[1]University California Irvine – Edwards Lifesciences Foundation Cardiovascular Innovation and Research Center; [2]University of Michigan; [3] University of California San Francisco; [4]University of Texas Southwestern; [5]University of South Carolina

Preterm birth is associated with significant right ventricular (RV) and pulmonary dysfunction, yet the myofilament-level mechanisms driving these changes remain largely unknown. Postnatal hyperoxia exposure (Hx; 85% O_2, 14 days) in term-born rat pups captures key features of human preterm birth, including RV dysfunction. We developed a subject-specific, multiscale computational model to investigate how myofilament-level changes contribute to RV dysfunction in preterm birth using the rat model in early life.

The multiscale model incorporates a five-state crossbridge kinetics model with calcium (Ca^{2+})-dependent activation and mitochondrial metabolite concentrations (ATP, ADP, inorganic phosphate) embedded within a myofiber mechanics model. This drives contraction and relaxation of the RV free wall, left ventricular (LV) free wall and septum within the TriSeg model, coupled to a reduced order closed-loop circulation model. Crossbridge parameters were informed by isolated RV trabeculae data from Hx and normoxic (Nx) rats at postnatal day (P21).

Patel et al. (2017) reported an approximately 70% increase in the maximum Ca^{2+} activated force, F_{max},at pCa = 4.5, and a leftward shift of the force-calcium curve at pCa_{50}, indicating an increased Ca^{2+}-sensitivity of force in Hx compared to Nx. To identify the crossbridge kinetic steps responsible, we simulated the force-Ca^{2+} experiment using a four-state strain-dependent sarcomere model at a fixed sarcomere length of 2.2 μm, varying Ca^{2+} concentration from pCa 7 to pCa 4.5. A total of 11 parameters increased F_{max} or pCa_{50} to reproduce the experimentally observed changes to the force-Ca^{2+}. However, five parameters had to be changed outside of physiological domains to achieve the experimentally observed change in pCa_{50} and F_{max}. Of the remaining six parameters, we decreased the Ca^{2+}-troponin C unbinding rate (k_{off}) and increased the crossbridge stiffness constants ($k_{stiff,1}$ and $k_{stiff,2}$) and successfully performed subject-specific model calibration for 19 animals (Nx: n=7, Hx: n=12) by minimizing residuals between simulated and measured biventricular pressure-volume waveforms.

While the imposed changes in k_{off}, $k_{stiff,1}$ and $k_{stiff,2}$ adequately modeled the changes in crossbridge kinetics and force that occur in this rat model of preterm birth, additional experiments are required to rule out or rule in other parameter changes that are consistent with the behavior.

Poster #96

The effect of NIV on the respiratory muscle pump in end-stage COPD.

Fleur van Brakel, PhD candidate Amsterdam UMC

Supervisor; C.A.C. Ottenheijm

To breathe effectively, one requires not only intact lungs but also strong respiratory muscles, known as "the respiratory muscle pump," to move air in and out. In 25% of patients with chronic obstructive pulmonary disease (COPD), the respiratory muscle pump fails, resulting in chronic hypercapnic respiratory failure (CHRF). Respiratory muscle pump failure is associated with a poor prognosis, and with a 2-years mortality rates of ~30%. A substantial and growing proportion of COPD patients with CHRF now receive chronic nocturnal non-invasive ventilation (NIV) at home. Chronic NIV may enhance health-related quality of life (HRQoL) and extend survival. However, not all patients experience benefits. In fact, over the course of treatment, patients can become progressively more reliant on the ventilator, which can rapidly deteriorate the patient's overall condition. In a very selected group of COPD patients, lung transplantation (LTx) is offered. LTx replaces the diseased lungs and has the potential to greatly improve HRQoL and survival. In cases where NIV is used as a "bridge to lung transplant," the decline in physical health may result in removal from the transplant waiting list, or, if transplantation occurs, a challenging and prolonged postoperative recovery period with intensive rehabilitation, and prolonged hospital admittance. We propose a novel concept in which these clinical observations are caused by chronic NIV-induced injury of the respiratory muscle pump. We have previously shown that invasive mechanical ventilation rapidly (within 2-3 days) causes respiratory muscle pump dysfunction, contributing to weaning failure (PMID: 39083588). Moreover NIV limits patient triggering of a breath, rendering the respiratory muscles inactive and thus highly susceptible to atrophy and dysfunction (PMID: 39137747). Pilot data indicates that diaphragm myofibers isolated from biopsies obtained during LTx have a severely impaired force response to Ca^{2+} in COPD patients compared to control patients, supporting our hypothesis.

Does Modifying Calcium Sensitivity Modify Mechanical Control (Strain Rate Sensitivity) of Relaxation?

Anita Abbo[1], Jia Yi Koh[1,2], Charles S Chung[1]
[1]Department of Physiology, Wayne State University, Detroit MI
[2]College of Osteopathic Medicine, Michigan State University, Detroit MI

Introduction: Detachment of myosin from actin is commonly assessed under steady calcium conditions, using single-molecule methods such as in vitro motility or permeabilized fibers, measuring the distortion response to a step stretch. We have previously shown that myocardial relaxation is sensitive to the rate of stretch imposed prior to or during the relaxation period. Our prior work has shown that strain-rate sensitivity, termed Mechanical Control of Relaxation, can be modified by varying preload and by some myosin-modulating drugs.

Goal: To determine if Levosimendan (Levo), an inodilator that modifies calcium sensitivity of the thin filament, modifies Mechanical Control of Relaxation (MCR).

Methods: Intact cardiac trabeculae were isolated from Sprague Dawley rats and randomized into treatment with increasing concentrations of Levo or equivalent doses of DMSO. Muscles were paced at 0.5 Hz at room temperature in a Tyrodes solution. MCR was assessed after 20 minutes of treatment (increasing dose of Levo). Data were analyzed for twitch parameters and MCR; Generalized Linear Mixed Models were used to assess the statistical significance of the effect and interaction of treatment (Levo vs DMSO) and dose for normalized data.

Results: There were no statistically significant differences in baseline measures of developed tension, time to peak, tension development rates, relaxation rates or MCR. Time to peak twitch and developed tension were reduced by increasing concentrations of Levo, a result we have observed previously using myosin modulating drugs. Neither single beat relaxation rate nor MCR were significantly dependent on treatment or dose.

Implications: These data, along with prior findings using OM and other compounds, suggest that calcium sensitivity itself is not a modifier of MCR. Cooperativity is still a potentially important modifier, as our prior work showed Omecamtiv Mecarbil modifies MCR but Danicamtiv does not. The complete mechanisms of Strain Rate sensitivity, especially in intact muscles, remains unresolved.

Preserved Baseline Diaphragm Function with Impaired Contractile Reserve Under Stress in HFpEF

Marloes van den Berg, MD, PhD[1]; Sem Ottenheijm, BSc[1]; Shanna Hamilton, PhD[1]; Henk Granzier, PhD[1]; Coen Ottenheijm, PhD[1]

[1]Department of Cellular and Molecular Medicine, College of Medicine – Tucson, University of Arizona, Tucson, AZ, USA

Background: Respiratory symptoms are highly prevalent in heart failure with preserved ejection fraction (HFpEF) and contribute to morbidity and hospitalization, yet the diaphragm remains understudied. Obese ZSF1 rats, a model of HFpEF, display preserved diaphragm function at baseline, suggesting compensatory adaptations that may fail under stress. We hypothesized that acute-on-chronic stress unmasks diaphragm dysfunction due to intrinsic myofilament alterations.

Methods: Lean and obese ZSF1 rats underwent in vivo diaphragm echography at 24 weeks of age to assess baseline structure and function. Acute-on-chronic stress was induced by intraperitoneal epinephrine/caffeine injection. After 45 minutes, diaphragm strips were harvested for ex vivo contractility studies, and tissue was collected for analysis of myosin light chain kinase 2 (MYLK2) expression.

Results: At baseline, obese ZSF1 rats demonstrated preserved diaphragm function compared with lean controls, characterized by increased diaphragm thickness, reduced zone of apposition length, and increased respiratory rate and contractility (all $p<0.01$). In contrast, acute stress unmasked impaired contractile reserve in obese rats, evidenced by a 40% reduction in maximal tension ($p<0.01$) and a trend towards shorter optimal diaphragm strip length ($p=0.052$). MYLK2 expression was reduced in the obese diaphragms ($p=0.03$), suggesting impaired regulatory light chain phosphorylation and altered cross-bridge kinetics, consistent with intrinsic myofilament dysfunction.

Conclusions: In HFpEF rats, diaphragm function is preserved at baseline but fails under stress, revealing an impaired contractile reserve. Myofilament alterations, including reduced MYLK2, likely contribute to this dysfunction and may underlie respiratory symptoms and exercise intolerance. Targeting diaphragm contractile reserve may represent a novel therapeutic avenue in HFpEF.

Poster #99

Myosin-Binding Protein C N-Terminal Domains Slow Ca^{2+} Dissociation from Cardiac Thin Filaments

Garrett T. Hauck[1,2], Fiona L. Wong[1], Matthew M. Klass[2], Victoria C. Lepak[1], Thomas A. Bunch[1], Jil C. Tardiff[2], and Brett A. Colson[1]

[1]Department of Cellular & Molecular Medicine, University of Arizona, Tucson, Arizona 85724

[2]Department of Biomedical Engineering, University of Arizona, Tucson, Arizona 85724

The cardiac thin filament (cTF) regulates contraction and relaxation through Ca^{2+} binding and release from cardiac troponin C (cTnC), yet how sarcomeric accessory proteins modulate these kinetics remains unclear. Cardiac myosin-binding protein C (cMyBP-C), anchored to the thick filament by its C-terminal domains, extends N-terminal domains that interact with both thin and thick filaments and are regulated by protein kinase A (PKA)-mediated phosphorylation. To determine how cMyBP-C influences Ca^{2+} exchange kinetics, we performed stopped-flow measurements using IAANS-labeled cTnC in reconstituted cTFs. The N-terminal fragments C0-C2 and C1-C2 slowed Ca^{2+} dissociation in a concentration-dependent manner, whereas phosphorylation markedly attenuated this effect. In contrast, C0-C1 had no impact, and none of the fragments altered Ca^{2+} association kinetics. Despite these functional differences, all fragments bound cTFs with similar stoichiometry (~1-2 cMyBP-C per tropomyosin), demonstrating that modulation of Ca^{2+} dissociation arises from specific functional domains rather than differences in binding. Consistent with this, cMyBP-C had no effect on Ca^{2+} dissociation from isolated troponin, indicating that this modulation of regulation requires an intact thin filament, likely through interactions with actin and/or tropomyosin. Slowing Ca^{2+} dissociation stabilizes the activated thin filament state, thereby delaying deactivation without altering Ca^{2+} association kinetics. These findings establish a previously unrecognized role for cMyBP-C in controlling Ca^{2+} dissociation from cTnC. We propose that cMyBP-C locally slows thin filament deactivation within the C-zone in a phosphorylation-dependent manner, thereby providing a mechanism to tune the timing of contraction and relaxation under varying physiological conditions.

Poster #100

Mechanosensitive Myopathy: FHL1 Missense Variants Impair Strain Sensing Response

Cara L. Barnett, Jordan R. Beach, Patrick W. Oakes

Loyola University Chicago

Cells are constantly subject to mechanical forces that challenge their ability to function. This is especially true in muscle, where high contractile forces propagate within and between cells. The actomyosin cytoskeleton is the dominant mechanical element, with actin filaments as semiflexible polymers that can bend and stretch, but with limits to physical perturbations. Previous studies identified LIM (Lin-11, Isl-1, Mec-3) domain proteins that recognize and stabilize strained actin. There are ~70 LIM domain genes in humans, and several cause genetic muscle diseases. The molecular mechanisms of these diseases and any contribution from actin strain sensing remain poorly understood.

FHL1 (four and a half LIM domains 1) is highly expressed in muscle, where it localizes to Z-discs and costameres. Clinically, *FHL1* missense variants are characterized by progressive hypertrophic cardiomyopathy and skeletal myopathy. We hypothesize that these diseases derive from impaired FHL1 mechanosensing. To test this, we used our established laser ablation assay in fibroblasts to induce an actin strain site and measure recruitment of wild-type FHL1 or pathogenic variants in the LIM domains (p.W138S, p.C225R, p.C240W, p.C292Y). Wild-type FHL1 was consistently recruited to actin strain sites, while all four missense variants demonstrated impaired recruitment. We next replicated this finding in neonatal rat ventricular cardiomyocytes (NRVMs) where induction of a stress fiber strain site at the Z-disc results in recruitment of wild-type FHL1.

While FHL1 expression is primarily cytoplasmic in fibroblasts, it localizes to adhesions and stress fibers when zyxin, another mechanosensitive LIM domain protein, is knocked out. FHL1 expression also increases dramatically when zyxin is knocked out. These findings suggest a model in which zyxin and FHL1 compete for binding to strained actin. We propose LIM domain protein competition for strained actin is likely also occurring in sarcomeres.

Our future goals include assessing mutant FHL1 response to strained actin at the Z-disc, and exploring competition between other Z-disc LIM domain proteins, such as LDB3. While the mechanisms for maintaining cytoskeletal integrity in muscle remains poorly defined, our findings suggest that impaired mechanosensing could lie at the heart of disease due to FHL1 and other mechanosensitive LIM domain proteins.

Poster #101

Protein Tyrosine Phosphatase 1B as a Novel Regulator of Myofilament Function in Disease

Authors: Door, M.M., Stachowski-Doll, M.J., Sun, Y., Pak, H., Hodgson, M., Sherer, L.A., Kontaridis, M.I., Kirk, J.A.

Phosphorylation of myofilament proteins regulates cardiac contractility and kinetics and is known to be dysregulated in heart failure (HF). Most studies have a primary focus on kinase dysregulation; however, phosphatases are relatively less understood. Inhibition of the ER-localized protein tyrosine phosphatase non-receptor type 1 (PTP1B) is a well-validated therapeutic target for cardiomyopathy. However, development of pharmacologic agents targeting PTP1B remain stunted due to poor understanding of PTP1B activity and substrates in cardiomyocytes.

Current literature supports that the cardioprotective effect of PTP1B inhibition is due to a pathological upregulation of PTP1B activity and expression under stress. Our data reflects this, where human left ventricular (LV) heart failure tissue has increased mRNA expression of PTP1B compared to non-failing rejected donor hearts. Similarly, mice subjected to ischemia-reperfusion injury (IR) have upregulated PTP1B protein expression. To better understand PTP1B targets in cardiomyocytes, we performed phospho-proteomics on LV tissue from constitutive cardiomyocyte-specific PTP1B-KO mice subjected to high-fat diet (known to upregulate PTP1B). Surprisingly, PTP1B-KO hearts had increased phosphorylation of myofilament proteins, including Troponin I (cTnI), Titin, and Myosin. How could an ER-tethered phosphatase be modulating myofilament proteins?

In human platelets that calpains can remove the ER tether sequence, resulting in a cytoplasmic PTP1B cleavage product (C-PTP1B) with double the activity of the full-length counterpart. We hypothesized this cleavage might be occurring in cardiomyocytes given PTP1B's sarcomere targets. Indeed, IR mice had a ~2.6-fold increase in C-PTP1B. We validated cytoplasmic localization of C-PTP1B via membrane fractionation and western blot of neonatal rat ventricular myocytes, validating translocation of PTP1B in cardiomyocytes. Additionally, proteomic analysis of membrane and cytosolic fractions of human explanted LV demonstrated PTP1B presence in the cytosolic fraction. No precise calpain cleavage site has been identified for PTP1B. We utilized PROSPERousPlus substrate cleavage prediction tool to predict calpain cleavage sites of PTP1B. Cross referencing these results to our human proteomic data, we identified a likely cleavage site at amino acid residues 383/384. Together, this data supports a role for PTP1B cleavage as a mechanism of myofilament regulation in disease.

To explore the functional significance of these targets, we treated a mouse model of heart failure with preserved ejection fraction (HFpEF, two-hit model) with a small molecule inhibitor of PTP1B (DPM-1001) or vehicle. We isolated single skinned myocytes from the LV from these hearts and assessed their function. DPM-1001 rescued length-dependent activation (LDA) and partially rescued titin-based passive stiffness in HFpEF. Corroborating our findings in PTP1B-KO mice, we found cTnI phosphorylation of S23/S24 was increased with pharmacological PTP1B inhibition. While this change would be expected to be an indirect effect of PTP1B inhibition, phosphorylation of S23/S24 cTnI plays a critical role in Ca2+ sensitivity and may, in part, explain the rescue in LDA. In all, this data identifies a novel mechanism for myofilament phosphorylation and contractile regulation by PTP1B.

Category: Myofilament-based Mechanosensing.

Poster #102

Titin M-band mechanosensing coordinates uterine peristalsis and embryo implantation

V Juhi Sur, Claudia Fink, Nora Bergmann, Rene Jüttner, Melissa Klingeberg, Fabian Coscia, Lorena Suarez-Artiles, Philipp Mertins, Johanna Elisabeth Fritsch, Melissa Birol, Maik Gollasch, Michael Gotthardt

Max Delbrück Center for Molecular Medicine (MDC), University Medicine Greifswald, Greifswald, and Charité – Universitätsmedizin Berlin, Berlin, Germany

Coordinated uterine peristalsis positions embryos for implantation, yet the pathways that synchronize contraction–relaxation cycles in the peri-implantation uterus remain incompletely defined. Titin is a central mechanosensitive scaffold in striated muscle, but its abundance, isoforms, and signaling roles in uterine smooth muscle have been uncertain. Here, we define a titin-dependent mechanotransduction program that controls peristalsis and embryo spacing.

Using smooth-muscle–specific deletion of titin M-band exons 1–2, ex vivo uterine ring myography and strip-based wall-motion tracking to quantify force and coordination, and *in vivo* implantation mapping during early pregnancy. We combined vertical agarose gel electrophoresis with mass spectrometry to identify uterine titin isoforms in myometrium and endometrium and validated the pathways involved with targeted immunoblotting.

The myometrium contained a truncated smooth-muscle titin isoform (smitin) and low-abundance full-length titin including the M-band kinase region. M-band deletion produced a reproductive phenotype with fewer implantation sites, mislocalization, and increased placental weight at term. Functionally, knockout uteri generated higher oxytocin-evoked peak force while passive wall tension was unchanged. In contrast to force gain, wall-motion tracking revealed fewer, spatially confined contraction peaks, indicating degraded coordination of myometrial waves.

Pure-myometrium proteomics identified a coherent signaling program consistent with hyperexcitability and altered tissue coupling: a proteostasis/redox reset, excitability and Ca^{2+} handling shifts, and ECM/adhesion remodeling alongside metabolic support. Whole-uterus immunoblotting showed a decrease in NBR1 with increased p62/SQSTM1 and reduced telokin and MLCK level, supporting impaired relaxation and Ca^{2+}-sensitization as proximal drivers of force gain.

Together, these data position the titin M-band as an autophagy–redox–excitability integrator that restrains uterine excitability and preserves wave coordination. Its disruption yields a hypercontractile yet poorly synchronized uterus, providing a mechanistic basis for embryo crowding and abnormal implantation spacing. The pathway highlights actionable targets (e.g., cGMP/telokin axis, SK3/ion handling, prostaglandin transport, and ECM signaling) to modulate peri-implantation motility with potential relevance to assisted reproduction.

Category: Myofilament-based Mechanosensing.

Poster #103

Effects of dextran in skeletal muscle with and without Myosin Binding Protein-C

Alexey V. Dvornikov[1], Nichlas M. Engels[2], and Samantha P. Harris[1]

[1]Department of Physiology, University of Arizona

[2]Department of Cellular and Molecular Medicine, University of Arizona

Myosin-binding protein C (MyBP-C) is an accessory sarcomeric protein which has been shown to form transverse C-links between thick and thin myofilaments. We previously reported that acute removal of MyBP-C N-terminal domains (C1-C7) decreased myofilament Ca^{2+} sensitivity of tension and increased lattice spacing in myofilaments from psoas muscle, consistent with activating effects of MyBP-C on the thin filament and it exerting a compressive effect on the sarcomere lattice. Here we tested whether osmotic compression of the myofilament lattice by dextran eliminates the rightward shift in the tension-pCa relationship observed upon the acute removal of MyBP-C N'-terminal domains.

We generated a mutant mouse line allowing for the quick cut of the N-terminal fragments (C1-C7) of both skeletal paralogs of MyBP-C following exposure of detergent-permeabilized muscle preparations to TEV protease (TEVp). We then measured force-pCa relationships and responses to a 2% increase in muscle length in TEV protease-treated and untreated psoas muscle fibers with and without 3% dextran.

Results showed that acute ablation of both paralogs of MyBP-C in the presence of 3% dextran tended to desensitize myofilaments to calcium as previously shown in our lab. However, the rightward shift in the presence of 3% dextran did not reach statistical significance, potentially due to our small sample size (from 5.57±0.02 to 5.52±0.03, n=4; p=0.11). Results also showed that responses to rapid stretch in fast-twitch fibers from psosas were characterized by an initial spike in force (P1) followed by a slower decrease in force to P2. Phase 3, a subsequent increase in force referred to as "stretch activation", sometimes occurred but was generally brief and less pronounced. However, TEVp treatment significantly altered the response to stretch by reducing P2 (from 0.04 to -0.004 mN, n=4; $p<0.001$), and revealing a P3 component of force. Similar effects of TEVp on stretch responses occurred in the presence of 3% dextran.

We conclude that dextran (and presumably lattice spacing) has minimal effect on the ability of MyBP-C to reduce Ca^{2+} sensitivity of tension or alter MyBP-C's effects on transient responses to stretch in fast twitch muscle.

Transmural Changes in In Vivo Myosin Motor Dynamics in a Murine Model of Truncated cMyBP-C Hypertrophic Cardiomyopathy

James T Pearson[1], Mark T Waddingham[1], Hirotsugu Tsuchimochi[1] and Vasco Sequeira[2].

[1]Cardiac Physiology, National Cerebral and Cardiovascular Center, Suita. Japan and [2]Comprehensive Heart Failure Center, Würzburg, Germany

Many studies have clearly shown that HCM associated with mutations in cardiac myosin binding protein-C (*MYBPC3*) are characterized by hyperdynamic contractions (hypercontractile), diastolic dysfunction and prolonged left ventricle (LV) relaxation. Myosin motors cyclically interact with actin filaments, forming and breaking cross-bridges (XBs) to generate the force necessary for contraction and relaxation in a process that is adjusted according to the heart's requirements. On a beat-to-beat basis, intracellular Ca^{2+} ion concentration, ATP availability, the load on the heart and many other factors govern XB formation, force generation and myofilament sliding (sarcomere shortening), and myosin deactivation for relaxation. Routinely, small-angle X-ray scattering (SAXS) equatorial reflections from isolated cardiac muscle are used to inform on structural myosin motor state transition from the inactive or ordered 'OFF' state to the active or disordered 'ON' state. SAXS studies on isolated muscle, including meridional reflection analyses, have been central to the development of models of cardiac muscle contraction cycles. However, it has also long been established by cardiac pressure-volumetry, echocardiography and magnetic resonance imaging that muscle work contributions to blood ejection varies regionally and across myocardial layers (transmural gradient). Our aim is to understand how myocardial layer specific, in vivo myosin motor dynamics are affected by HCM due to a truncation mutation in *Mybpc3*, which results in cMyBP-C deficiency in the myofilaments.

Truncation mutant *Mybpc3* (Exon 33 del/del, HCM) mice were generated by CRISPR/Cas9 deletion of exon 33 on C57Bl/6N background and standard echocardiography assessments made. Adult mice were then surgically prepared under isoflurane anesthesia (2-4%) at the Japanese Synchrotron facility SPring-8 for in vivo SAXS recordings simultaneous with LV pressure-volumetry to compare transmural myosin motor function with global cardiac function. The X-ray microbeam was passed through the LV from the epicardium to subendocardium in a sequential series (10ms intervals, ~2s each projection) to obtain baseline recordings and then recordings were repeated 5min after intraperitoneal administration of dobutamine hydrochloride. Mice were then euthanised with KCl during isoflurane inhalation and hearts removed for further analysis. Integrated intensities of myosin 1,0 and actin-myosin 1,1 reflections were determined as an intensity ratio ($I_{1,0}$ / $I_{1,1}$) for each time point across the cardiac cycle to estimate myosin head XB formation, changes in ON/OFF state and $d_{1,0}$ spacing (interfilament spacing inversely related to sarcomere length). Relative to WT mice, HCM mice were found to have reduced ejection fractions, increased E/e' (filling pressure) and prolonged relaxation, in addition to reduced LV global longitudinal and circumferential strain, consistent with similar *Mybpc3* mutant models. In the HCM mice a greater proportion of myosin heads remained in an ON state in diastole, with less XB formation during the ejection phase and myosin interfilament spacing increase during systole was very reduced, suggesting that sarcomere shortening was impaired. A persistent low intensity ratio during filling implies a failure of myosin heads to deactivate in late diastole, especially in the subendocardium. In WT hearts myosin deactivation during diastole was enhanced by dobutamine, whereas in del/del hearts dobutamine did not improve detachment in the subendocardium and in fact, sustained the ON state for most of diastole in the epicardium. Our analyses also reveal an uncoupling of myosin activation from myofilament sliding (shortening) and normal blood ejection associated with cMyBP-C deficiency in the sarcomeres in advanced stages of HCM.

Loss of Myosin binding protein-C (MyBP-C) increases strain induced cross-bridge detachment in striated muscle

Nichlas M. Engels[1], Rachel L. Sadler[2], Anthony L. Hessel[3], Samantha P. Harris[2]*

1. Cellular and Molecular Medicine, University of Arizona, Tucson, USA. 2. Department of Physiology, University of Arizona, Tucson, USA. 3. Institute of Physiology II, University of Muenster, Germany.

Myosin binding protein-C (MyBP-C) is a family of regulatory protein located in cardiac and skeletal muscle sarcomeres. It's N-terminal domains are critical in regulating both thick and thin filament activation. MyBP-C is able to stabilize the OFF state of myosin heads, while during Ca^{2+} activation it shifts the tropomyosin cable on the thin filament from the blocked to the closed state, revealing binding sites for myosin. The MyBP-C family consists of three proteins, each encoded by a unique gene: *Mybpc1* encodes the slow-skeletal, *Mybpc2* encodes the fast-skeletal, and *Mybpc3* encodes the cardiac paralog of MyBP-C. Although each protein share similar structural homology, the sequence identity between each protein lies around 40% in humans. Therefore, although each protein is thought to contribute similar effects to muscle function, significant differences may exist in each paralogs regulatory function. Here, we sought to identify the functional differences between each MyBP-C paralog following a rapid stretch protocol, which is a measure of crossbridge attachment and detachment. In order to test this, we used three mouse lines termed SpyC1 for slow-skeletal, SnoopC2 for fast-skeletal, and SpyC3 for cardiac muscle. Each mouse line contains a TEV protease recognition site between domains C7-C8, allowing for removal of domains C0-C7 (C1-C7 in skeletal muscle) following TEVp incubation. SpyC1 and SpyC3 contain a SpyTag between C7-C8, while SnoopC2 contains a SnoopTag. Both the SpyTag and SnoopTag form a spontaneous covalent bond with a recombinant MyBP-C containing either a SpyCatcher or a SnoopCatcher domain, respectively. Therefore, these mouse lines allow for *in situ* replacement of recombinant MyBP-C within the muscle sarcomere. We found that removal of MyBP-C increased the rate of relaxation as well as decreased the P2 amplitude in each muscle type, indicating increased cross-bridge sensitivity to strain. Furthermore, addition of slow-, fast-, and cardiac MyBP-C into SpyC3 left ventricle tissue decreased relaxation and increased the P2 amplitude, showing that the presence of each paralog was able to decrease cross-bridge sensitivity to strain in cardiac sarcomeres. This suggests that the presence of MyBP-C stabilizes the cross-bridge, either through maintaining activation of the thin filament or stabilizing the bound cross-bridges during a rapid stretch maneuver.

Poster #106

H251N β-Cardiac Myosin Mutation Alters Cardiomyocyte Cell-Matrix Force Transmission via Focal Adhesion Remodeling In a Stiffness-Dependent Manner

Georgina A. Stephanie[1], Mari Park[2], Nathan B. French[2], Mahadev Josyabhatla[2], Alison Vander Roest, Ph.D.[2]

1. Department of Chemical Engineering, University of Michigan
2. Department of Biomedical Engineering, University of Michigan

Hypertrophic cardiomyopathy (HCM)–associated mutations in MYH7 alter sarcomere mechanics, yet how these mutations influence mechanobiological signaling in cardiomyocytes (CMs) remains poorly understood. The early-onset H251N β-cardiac myosin mutation increases actin motility, intrinsic force, and ATPase activity, consistent with a hypercontractile molecular phenotype. Here, we investigate the transmission of these forces from the cell to the matrix. We hypothesized that H251N-associated increases in molecular motor activity would enhance focal adhesion (FA) organization and mechanobiological responses. Human induced pluripotent stem cell-derived CMs (hiPSC-CMs) carrying the H251N mutation and isogenic controls were seeded onto deformable substrates to mimic phyhsiological and pathological stiffness. A Förster resonance energy transfer (FRET)- based molecular tension sensor was employed to assess intracellular FA organization and adhesion-associated tension. Our results reveal substrate-mediated FA remodelling. Compared to isogenic controls, H251N hiPSC-CMs cultured on soft substrates exhibited a lower FRET index, consistent with our hypothesis of enhanced tension in the H251N mutation at baseline conditions. Interestingly, we observed a shift in donor intensity on stiff substrates, suggesting sensitized focal adhesion turnover in the H251N hiPSC-CMs as substrate mechanics are altered. Our results show that H251N-associated increases in molecular motor activity are linked to stiffness-dependent alterations in FA remodeling and mechanotransductive coupling. This finding supports our hypothesis that the H251N myosin mutation promotes mechanosensitive remodelling through cell substrate adhesion complexes.

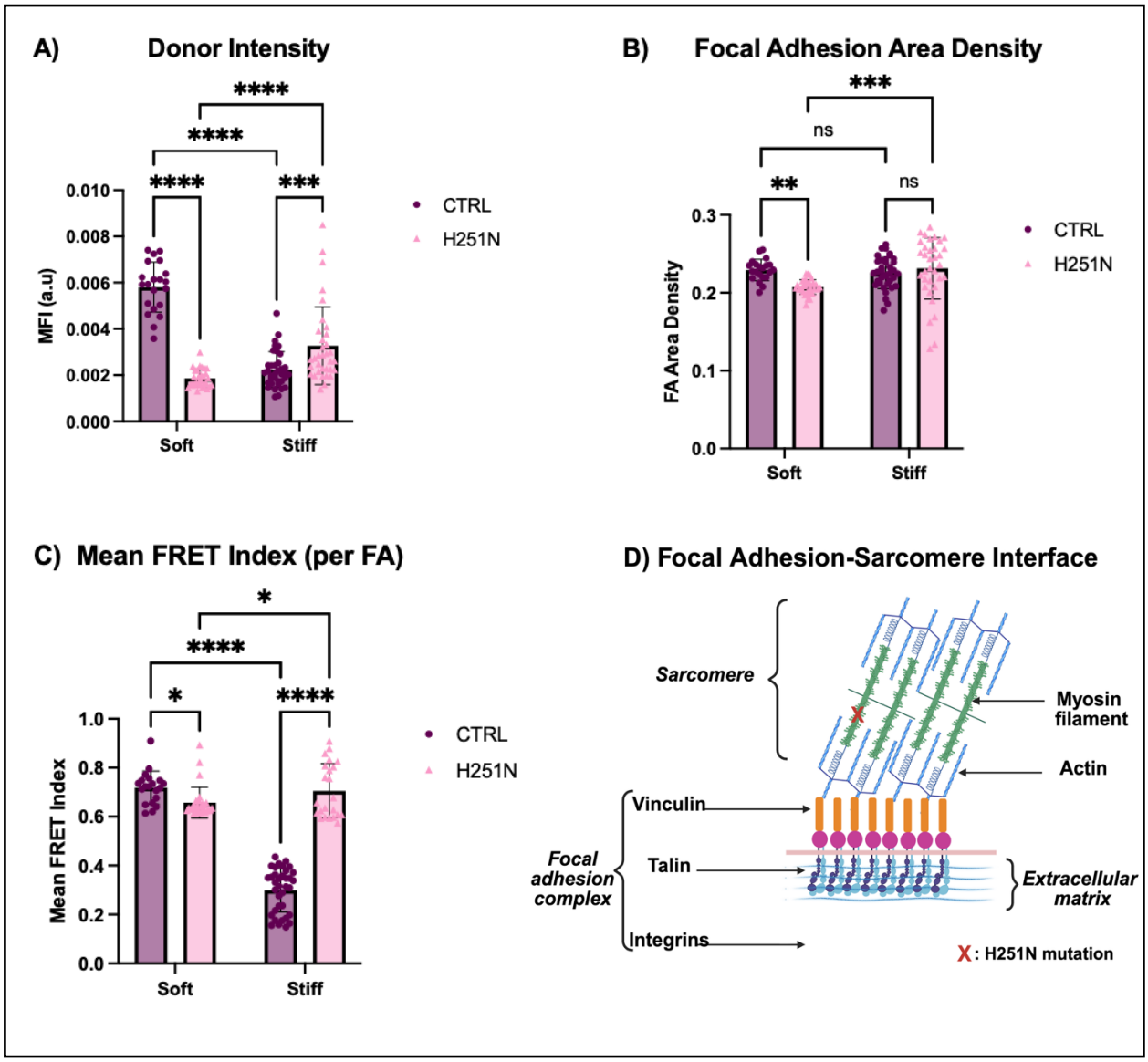

Figure 1. H251N induces stiffness-dependent changes in focal adhesion organization and mechanobiological signaling. A) Donor fluorescence intensity (MFI), B) FRET index, and C) focal adhesion (FA) area density in hiPSC-CMs cultured on soft (10 kPa) and stiff (60 kPa) substrates. D) Schematic of the focal adhesion–sarcomere interface. Two-way ANOVA revealed a significant interaction between genotype and substrate stiffness for donor fluorescence intensity ($p < 0.0001$), FRET index ($p = 0.0121$), and FA area density ($p < 0.0001$), indicating a stiffness-dependent effect of the H251N mutation. Consistent with this interaction, H251N cells exhibited lower donor intensity, FRET index, and FA area density relative to control on soft substrates, but higher values on stiff substrates. Data are presented as mean ± SD with individual cells overlaid; statistical significance indicated as shown.

Poster #107

Effects of Myosin-Specific Pharmacologics on Cardiomyocyte Mechanical Behavior

Nathan French[1], Alison Vander Roest[1] PhD
[1]University of Michigan, Biomedical Engineering
Category: Regulation and Modulation of Contraction | Consideration: Poster Presentation

Introduction

Common hallmarks shared amongst multiple types of heart disease includes changes in the myofibril architecture and post-translational modifications of cytoskeletal proteins. This work intends to investigate the contractile behavior and cytoskeletal protein abundance in cardiomyocytes treated with myosin-specific pharmacologics.

Materials and Methods

Human induced pluripotent stem cells (hiPSC) with an endogenous green fluorescent protein (GFP) reporter for α-actinin were differentiated into cardiomyocytes. iPSCs had been previously CRISPR edited to introduce known disease-causing β-Myosin (HCM-causing H251N; DCM-causing R369Q). On day 30 of the differentiation, cells were plated onto micropatterned 10 kPa polyacrylamide (PA) gels doped with fluorescent microspheres. Beating cardiomyocytes were imaged in brightfield and simultaneous dual-channel GFP/RFP imaging at 30 frames per second using the Hamamatsu W-View Gemini. Cardiomyocytes were treated using myosin modulators (Mavacamten, Danicamtiv, Omecamtiv Mecarbil) at 300nM concentrations; DMSO was used as the loading control for the study. Videos were captured at various timepoints (a) before, (b) 5 minutes, and (c) 24hr to study short-term and long-term effects of myosin modulation. Afterwards, the samples were stained for α-Tubulin and detyrosinated-Tubulin for visualization of cytoskeletal architecture.

Results, Discussion, and Conclusions

The location and type of amino acid substitution on the β-Myosin Heavy Chain (β-MHC) have significant effects on the functionality of myosin. Here it was demonstrated that the H251N mutation matches expectations by producing cells of higher overall contractile force, however, the R369Q mutation resulted in an unexpected higher contractile force (**Figure 1**). Longitudinal assessment of cardiomyocytes at three timepoints demonstrated the genotype-specific response to the pharmacologic, with the H251N mutation demonstrating some resistance to initial acute contractility changes, and movement back towards baseline contractile force in the 24hr conditions (**Figure 2**). Comparing the relative abundance of cytoskeletal proteins from immunofluorescence analysis (**Figure 3**) reveals a significant difference ($p=0.0002$) between the H251N and R369Q mutations with respect to the ratio of detyrosinated-tubulin to alpha-tubulin indicating genotype-specific changes in post-translational modifications of the cytoskeletal proteins.

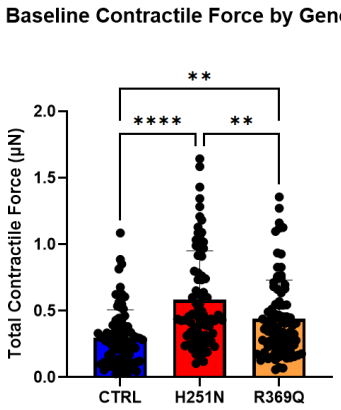

Figure 1: Traction Force Microscopy contractility analysis reveals genotype-induced hypercontractility by the H251N mutation

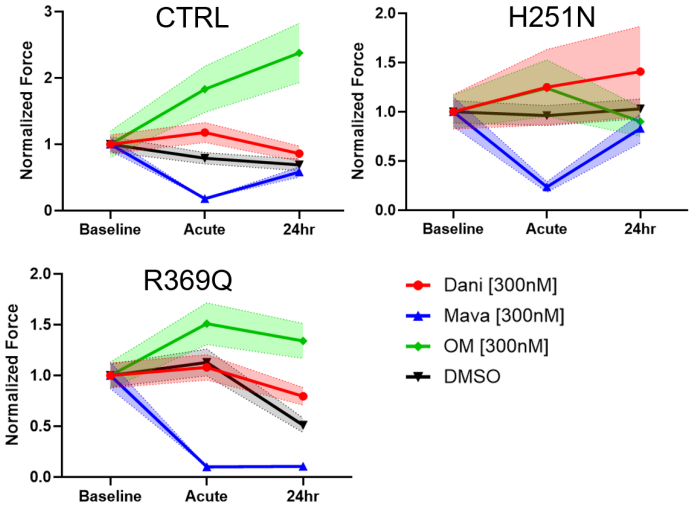

Figure 2: Longitudinal tracking of cardiomyocyte contractility at baseline, acute (5 minutes), and 24hr after pharmacologic exposure, with multiple drug comparison.

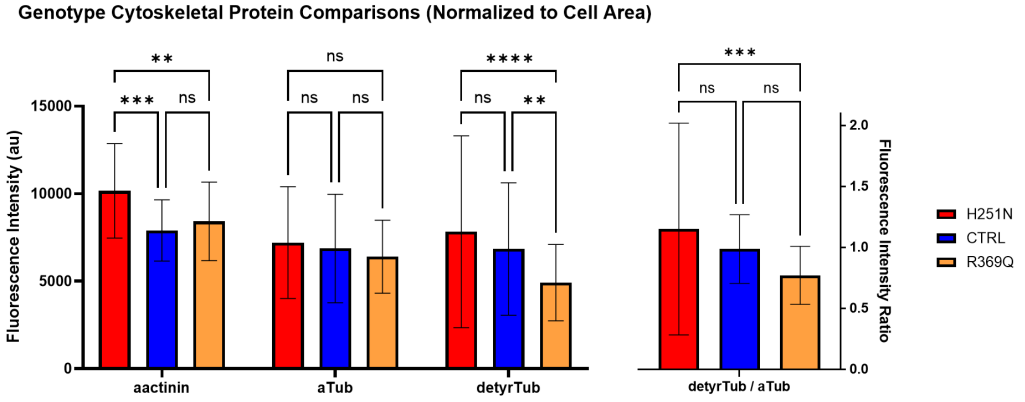

Figure 3: Immunofluorescence comparison of various cytoskeletal proteins (aactinin, aTub), post-translational modifications (detyrTub), and their ratios (detyrTub / aTub).

Poster #108

Effects of omecamtiv mecarbil on cross-bridge kinetics and power output in rat skeletal muscle fibers

Bertrand C.W. Tanner[1], Austin Au[1], and David C. Lin[1,2]

[1]Department of Integrative Physiology and Neuroscience, [2]Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, WA, United States. [3]Center for Translational Muscle Research, University of Washington, Seattle, Washington.

We have been investigating changes in myosin cross-bridge kinetics as sarcomere length varies during isometric contraction. Most of our prior studies have utilized permeabilized (skinned) soleus (slow-twitch skeletal) fibers or myocardial strips from mice and rats. In general, these studies have shown that cross-bridge detachment rate slows as sarcomere length increases—but we have not investigated length-dependent kinetics in fast-twitch skeletal muscle fibers. In addition, we were interested in testing whether omecamtiv mecarbil (OM) may be a useful tool for eliminating length-dependent myosin kinetics. OM is a myosin activator that was developed as a potential therapeutic to augment cardiac force production in patients with heart failure that show reduced systolic function, but OM has also been shown to slow myosin cross-bridge cycling largely due to slowed cross-bridge detachment. To test these ideas, permeabilized skeletal fibers from rat soleus (slow twitch) and medial gastrocnemius (MG=fast-twitch) muscles were Ca^{2+}-activated (pCa 4.0) at short (2.4 μm) and long (2.7 μm) sarcomere lengths. We used step-length perturbation analysis to assess cross-bridge kinetics in the presence and absence of OM. Without OM, cross-bridge detachment rates slowed as sarcomere length increased for soleus fibers, but not for MG fibers. With 1 μM OM, cross-bridge detachment rates slowed for soleus fibers and length-dependent kinetics remained slower at long sarcomere length. In contrast, 1 μM OM did not affect cross-bridge detachment rates and there was no effect of sarcomere length for MG fibers. In a sub-set of fibers we performed force-velocity measurements at 2.7 μm sarcomere length, where again, OM slowed shortening velocity and diminished power output for soleus fibers, but not for MG fibers. Thus, sarcomere length and OM slowed cross-bridge kinetics for slow-twitch, but not fast-twitch skeletal muscle fibers. Building on our prior findings, these data suggest that slow-skeletal and cardiac myosin isoforms may be more sensitive to changes in sarcomere length than fast-skeletal myosin isoforms. Moreover, OM may only affect slower myosins, possibly due to OM not binding to fast-skeletal myosins.

Poster #109

Altered contractile dynamics underlie adaptations in cardiac function in the maturing porcine heart

Lauren Gilbertsen, Abigail Thomas, Julianne Smith, and Daniel Fitzsimons

Department of Animal, Veterinary, and Food Sciences

University of Idaho, Moscow ID 83844

The cardiopulmonary transition from fetal to neonatal circulation poses a formidable cardiovascular challenge at birth. At full-term, the newborn heart is characterized by reduced left ventricular mass (as a percentage of total heart mass), reduced Ca^{2+} handling, low mitochondrial density, incomplete myofibrillar organization, and impaired left ventricular filling due to low ventricular compliance. These functional deficiencies are rapidly corrected within the first few months post-partum and are accompanied by the well-documented troponin complex isoform switch from fetal (ssTnI-TnT_1-cTnC) to adult (cTnI-TnT_3-cTnC). In the mammalian heart, the generation of contractile force involves the synergistic activation of the myocardial thin filament by Ca^{2+} and strong binding cross-bridges. We propose a model that thin filament responsiveness to the activating effects of Ca^{2+} and myosin cross-bridge binding stratifies with the developmental up-regulation of the adult troponin complex expression, and thereby progressively improve beat-to-beat ventricular function. To test this idea, we examined the Ca^{2+}-dependencies of force and the rate of force redevelopment (*k*tr) in permeabilized myocardium isolated from porcine neonatal hearts at day-3 (d3) and day-30 (d30) post-partum. Compared to d30 myocardium, the d3 myocardium exhibited an increase in the Ca^{2+}-sensitivity of force, i.e., the pCa_{50} increased from 5.66 ± 0.02 to 5.77 ± 0.02 (ΔpCa50 = 0.11 ± 0.02). Furthermore, while the d3 myocardium exhibited a steep activation-dependence of the rate of force redevelopment, the activation-dependent profile of *k*tr was reduced in d30 myocardium. Western blot and phosphoprotein gel analyses were used to confirm the expression of troponin isoforms and the basal level of cTnI and cMyBP-C phosphorylation in d3 and d30 myocardium. These results can be explained, at least in part, in terms of a model in which troponin isoform switching modulates the extent and rate of cooperative spread of myosin cross-bridge binding to the myocardial thin filament.

Drosophila Aging Mirrors Human Sarcopenia: Age-Related Decline in Muscle Power and Mobility

Casey M. Ahlin, Alon T. Brown, Brian H. Jensen, Chloe C. Spitzer and Douglas M. Swank

The age-related decline in muscle function, known as sarcopenia, is characterized by reduced force, power, and endurance, which significantly impairs mobility and reduces quality of life. We are evaluating *Drosophila*'s potential as a model to understand the mechanisms behind human sarcopenia and elucidate ways to blunt its impact on human health. A previous study suggested that *Drosophila* indirect flight muscle (IFM) power is higher in older than younger flies, contrary to human muscles where loss of power output with age is well established. We questioned the counterintuitive *Drosophila* result and instead hypothesize that age-related changes will diminish IFM muscle performance. To test our hypothesis, we analyzed flight ability, wing beat frequency (WBF) and permeabilized single IFM fibers from adult wild-type (W^{1118}) flies at 7 (young), 28 (median), and 49 (aged) days post eclosion. Mechanical properties were characterized using sinusoidal analysis and work-loop perturbations to assess power output at small-strain and physiological length-change amplitudes. Sinusoidal analysis revealed a large reduction in maximum muscle power at 49 days, with a 72% decrease in males and a 76% decrease in females compared to 7-day old flies. Work-loop analysis mirrored these results, with oscillatory power output dropping by ~70% in both sexes. No significant IFM power decline was observed at 28 days compared to 7-days, suggesting full muscle function maintenance through mid-life and a sharp late life reduction. Active IFM stiffness (elastic modulus) fell significantly by day 49, decreasing by 77% in males and 58% in females, indicating decreased fiber stiffness and/or crossbridge binding. Flight ability was significantly impaired in aged flies with flight index decreasing 3-fold in both sexes compared to young flies. WBF analysis of aged flies that were able to beat their wings (45% of flies tested), showed a significant decrease in females by 16% at 49 days, with males displaying no decline. Our results show that *Drosophila* IFMs undergo significant age-dependent muscle mechanical declines that parallel human sarcopenia. Based on our results, *Drosophila* will serve as an excellent model for discovering the mechanisms behind sarcopenia and therapeutics to preserve muscle function.

Resolving ATP Turnover Kinetics and IHM Formation of β-Cardiac Myosin at the Single-Molecule Level

Rama Reddy Goluguri[1,2], Abrar Bhat[3], Alexander R Dunn[3],

James A Spudich[1, 2], Kathleen M Ruppel[1,2,4]

1. Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA
2. Stanford Cardiovascular Institute, Stanford, CA, USA
3. Department of Chemical Engineering, Stanford University, Palo Alto, CA, USA
4. Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA

Shane Nelson[5], David M Warshaw[5]

5. Department of Molecular Physiology and Biophysics, Cardiovascular Research Institute, University of Vermont, Burlington, VT, USA

Jinghua Ge[6], Christopher M Yengo[6]

6. Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA, USA.

β-cardiac myosin is a molecular motor that drives ventricular contraction. Under relaxing conditions, cardiac myosin can adopt a biochemical state that turns over ATP ~1000-fold more slowly than the actin-activated state. This super-relaxed (SRX) state plays a critical role in energy conservation and has been shown to be destabilized by multiple hypertrophic cardiomyopathy (HCM) causing mutations in cardiac myosin. The SRX state is typically estimated using a bulk single-turnover (STO) assay with fluorescent mant-ATP. However, this ensemble approach provides limited insight into the molecular details underlying the formation and decay of the SRX state and is complicated by experimental artifacts and choice of model used for interpreting the kinetic trace. To overcome these limitations, we have initiated a multi lab collaborative effort to directly observe myosin ATP turnover at the single-molecule level using TIRF microscopy-based assays. In this approach, myosin, with a fluorescent tag that can be used as a donor/acceptor fluorophore for fluorescence resonance energy transfer (FRET) or as a fiducial marker for locating myosin, is tethered to a microscope slide at picomolar concentrations, and nanomolar concentrations of fluorescent ATP are introduced. Myosin and ATP are imaged in separate channels either by exciting the label on myosin and fluorescent ATP directly with different lasers or indirectly through FRET by exciting the donor. Experiments in the Warshaw lab have already produced exciting results to meet our common goal of understanding the structural origin of the SRX state by performing single molecule FRET experiments with FRET sensors developed/made in the Yengo lab. Those results will be discussed by Shane Nelson in his talk Sunday afternoon. We at Stanford are performing complementary single molecule experiments by directly exciting fluorescent ATP bound to myosin. The dwell times of individual fluorescent ATP binding events are used to quantify the fraction of myosin molecules in the SRX state and single molecule fluorescence intensity time traces are analyzed to obtain mechanistic details of SRX formation and dissociation. In our collaborative experiments with the Warshaw and Yengo laboratories, the ultimate goal is to understand the intricate link between the biochemical SRX state and the structural IHM state.

Viral Vector Core Facility: accelerate your research with our reliable viruses

Olga V. Alekhina, Hendrikus L Granzier

Viral Vector Core Facility, University of Arizona

Viral vectors are essential tools for gene delivery in biomedical research, particularly in studies of cardiac biology, muscle function, and disease modeling. To facilitate these studies in the University of Arizona, Molecular Cardiovascular Research Program, we established a Viral Vector Core Facility that provides services for the design, production, and validation of viral vectors.

The core specializes in the production of adeno-associated virus (AAV) and adenoviral vectors, offering multiple serotypes and customizable constructs tailored to specific experimental needs. Our streamlined workflow includes construct design and cloning, viral production, purification, and rigorous quality control, including titration and validation. Standardized protocols ensure high reproducibility and consistent generation of high-titer viral preparations suitable for both in vitro and in vivo applications.

A major focus of the facility is supporting cardiovascular research. In particular, the core provides viral vectors optimized for cardiac and skeletal muscle studies, including MyoAAV variants for efficient muscle transduction.

By delivering reliable viral vectors and expert support for low prices, the Viral Vector Core Facility enables investigators not only from University of Arizona, but also from outside, to accelerate research while reducing the time and resources required for in-house production.

Modeling Cooperative Cross-Bridge Dynamics in Beating Heart

Nicholas Stahr[1], Joshua A. Hale[1,2], Sakthivel Sadayappan[2], Carl Tong[2,3]

[1] Texas A&M University, College of Medicine, Department of Medical Physiology.

[2] University of Arizona, College of Medicine, Department of Cellular and Molecular Medicine.

[3] University of Arizona, College of Medicine, Department of Medicine.

Background: Cross-bridge (CB) cycling follows a very complex process involving both thick and thin filament interactions for each heartbeat. Despite extensive experimental investigation, the integrated regulatory mechanisms governing these interactions remain incompletely understood. We hypothesized that a unified thick–thin filament cross-bridge (UTFCB) model could accurately reproduce experimentally observed contractile behavior.

Methods: We developed UTFCB model with MATLAB/Simulink simulation based on established biophysical principles and prior experimental findings. First-order differential equations with rate accelerators to capture dynamic cooperative effects modeled state transitions. The model was validated using simultaneously measured intracellular calcium and force data from intact ex vivo papillary muscle preparations.

Results: Calcium binding to troponin C transitions tropomyosin (Tm) from the blocked to the off state, exposing the actin filament. This allows myosin in the M·ADP·Pi state to weakly bind to actin. Transition to the strongly bound (CB-Strong) state further displaces Tm into the open state, exposing additional actin binding sites and promoting cooperative recruitment of more myosin heads. CB progresses to the rigor state following the release of ADP. Binding of ATP to rigor CB triggers its detachment from actin, resetting the myosin head into the resting M-ATP conformation. ATP is then hydrolyzed to regenerate the M·ADP·Pi state, priming the myosin for another cycle. If calcium is removed before myosin can rebind to actin, Tm transitions back to the blocked state to complete relaxation. Key regulatory features include: calcium initiates, cooperative enhancement of Tm-Open by strongly bound CB states, constants need to change with increasing heart rate, and CB cooperatively detaches followed by transition of myosin into the resting state independently of the thin filament status.

Conclusion: UTFCB simulation successfully reproduced experimentally observed calcium–force relationships of intact cardiac muscle, supporting its validity. This integrative model provides new mechanistic insight into cooperative regulation of cross-bridge cycling and offers a valuable platform for investigating alterations in cardiac contractility.

The replacement kinetics of the giant muscle protein nebulin are slow

Sylvia Bogaards[1], Michaela Yuen[1], Yeszamin Onderwater[1], Carlos Clara[1], Ricardo Galli[1], Miguel Vizoso[4], Stefan Conijn[2], Eva Peters[2], Leila Nahidiazar[1,3], Nimo Annor[2], Julia Muldoon[2], Neil B Wodd[5], Michael J Previs [5], Kees Jalink[3], Jacco van Rheenen[4], Henk Granzier[2], and Coen Ottenheijm[1,2]

[1]Department of Physiology, Amsterdam UMC, Amsterdam, the Netherlands; [2]Cellular and Molecular Medicine, University of Arizona, Tucson, USA; [3]Department of Cell Biology, Van Leeuwenhoek Centre for Advanced Microscopy, [4]Department of Molecular Pathology, Oncode Institute, The Netherlands Cancer Institute, Amsterdam, the Netherlands, and; [5]Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, Vermont, USA.

To date, it is unknown how giant muscle proteins are integrated and replaced in continuously contracting muscles. A prime example is the thin filament-associated, sarcomeric protein nebulin (~800 kDa), variants in which cause nemaline myopathy. To establish the replacement kinetics of nebulin in sarcomeres, we designed a mouse model with fluorescent Dendra2 inserted in nebulin's N-terminus (NebN-D2). Dendra2 was well tolerated by the sarcomere, as body weight, weight, force and structure of EDL and soleus muscles, and nebulin protein levels were similar to WT mice. *Intra-vital* (imaging window on *m. gluteus maximus*) or *ex vivo* FRAP microscopy on isolated FDB fibers was used to determine the replacement kinetics of nebulin, where Dendra2 was converted from green to red fluorescent state and fluorescence intensity was followed over time. Once Dendra2 is red, it cannot revert back to green, so an increase in green signal is due to replacement with new protein. Both *intra-vital* and *ex vivo* data indicate that nebulin's replacement kinetics are very slow (~2.5%/day and ~1.7%/day, respectively) after a quick mobility phase in the first minutes (10±4% and 14±3%, respectively; mobile fraction). During *intra-vital* imaging, after 19 days, ~half of the converted Dendra2 was still present in sarcomeres.

Using PALM (PhotoActivated Localization Microscopy) the location of the Dendra2-tagged nebulin was determined with nanometer resolution in fixed samples. The results showed that 14±4% of the molecules were not incorporated into the sarcomere of EDL muscle, supporting the mobile fraction measured with FRAP.

To quantify the nebulin turnover, we fed WT mice a diet in which 99% of the leucine contains 3 deuterium atoms and tracked the incorporation of this amino acid into nebulin using high resolution mass spectrometry. Data showed that the half-life of nebulin in EDL and soleus muscle is 35±2 vs 19±1 days. Thus, turnover rates closely match the replacement kinetics, suggesting that nebulin molecules do not reincorporated in other thin filaments.

An autosomal dominant mutation in nebulin that deletes 76 exons ($Neb^{\Delta ex14\text{-}89}$, lacking 17 of the 29 super repeats), was identified in a three-generation family. Although both WT and mutant nebulin proteins are expressed, our pilot data from patient myofibers reveal a striking predominance of the short, mutant isoform within thin filaments. This unexpected dominance may result from slower replacement kinetics of the short isoform and an increased cytosolic availability relative to the WT protein. We have designed a mouse model with Dendra2 tagged to the mutated nebulin ($NebN\text{-}D2^{\Delta ex14\text{-}89}$), these mice produce a shorter nebulin (lacking 17 of the 25 super repeats) and incorporate nebulin into the sarcomeres. Heterozygous mice show a predominance of the short nebulin, similar to patients. We will investigate the replacement kinetics of wt vs mutant nebulin in heterozygous mice.

Summarizing, our data indicate that the replacement kinetics of the giant protein nebulin in mature muscle are very slow.

Calcium-Independent Relaxation Enhancement is Impaired in Hearts with Diastolic Dysfunction

Joshua A. Hale[1,2], Sakthivel Sadayappan[1], Carl Tong[1,3]

[1]University of Arizona, College of Medicine, Department of Cellular and Molecular Medicine.

[2] Texas A&M University, College of Medicine, Department of Medical Physiology.

[3]University of Arizona, College of Medicine, Department of Medicine

Background: Although calcium-force coupling is well characterized in *ex-vivo* and *in-vitro* heart preparations, the coupling dynamics within intact *in-vivo* hearts are less characterized, particularly in the context of diastolic dysfunction. We hypothesize that calcium-independent regulation of cross-bridge detachment (relaxation) is altered with diastolic dysfunction such as Heart Failure with Preserved Ejection Fraction.

Methods: To elucidate calcium-force coupling dynamics of intact heart, we developed a system to perform *in-vivo* measurements of intracardiac pressure and intracellular calcium $[Ca2+]_i$ on mice expressing the cardiomyocyte-specific genetically encoded $[Ca2+]_i$ indicator GCaMP-8. These mice demonstrated similar diastolic function to wild-type (E/e' 22.9 ± 3.2 vs. 21.9 ± 3.7, p=0.546), making them an excellent platform for inducible diastolic dysfunction studies. We induced diastolic dysfunction using 15 weeks of simultaneous high-fat diet and L-Arganine nitric oxide synthase inhibitor (L-NAME) in drinking water. Replicate measurements (5) with >50 heartbeats were taken at baseline and following intraperitoneal injection of 10 ug/g bodyweight dobutamine for (n=3) High-Fat Diet+L-NAME (HFDL) mice and (n=6) control mice.

Results: HFDL mice showed significant reduction in $\left(\left(\frac{dP}{dt}\right)_{min} / \left(\frac{dP}{dt}\right)_{max}\right)$ at basal conditions (0.6590 ± 0.0972 vs. control 0.8844 ± 0.0955, p=0.0128) confirming diastolic dysfunction. HFDL hearts also demonstrated faster $[Ca2+]_I$ decay rates (decay slope -1.7565 ± 0.1284 vs. control -1.4671 ± 0.0924, p=0.0056) and shorter 75% decay time (0.4809 ± 0.0048 cycles vs control 0.5938 ± 0.0676 cycles, p=0.0269) at basal conditions. Following treatment with dobutamine, neither group showed significant changes to Ca^{2+} cycling parameters, including 50% pulse-width, 75% decay time, and decay slope. However, control hearts demonstrated significant narrowing of pressure pulse-width relative to heart period (0.3347 ± 0.0311 cycles vs basal 0.3895 ± 0.04897 cycles, p=0.01). This calcium independent dobutamine induced pulse pressure narrowing was not detected in HFDL mice.

Conclusion: These results indicate: (1) calcium independent pathogenic mechanism(s) caused diastolic dysfunction in HFDL, (2) ability to accelerate Ca^{2+} reuptake is blunted in HFDL, and (3) HFDL caused the loss of calcium-independent β-1 adrenergic mechanism to enhance relaxation. Furthermore, our experimental platform can be used to elucidate mechanisms responsible for diastolic dysfunction and guide development of treatments to restore diastolic function.

Flying into the Future of Sarcopenia: A Proteomic Atlas of Aging *Drosophila melanogaster* IFMs Supports Time-Dependent Molecular and Structural Dysregulation of Skeletal Muscle

Christine E. Delligatti[1], Rohan Wishard[1], Indira Paddibhatla[1], Sogol Sedighi[1], Alireza Khoddam[2], Weikang Ma[3], Thomas Irving[3], D. Brian Foster[1], Douglas E. Vaughan[2], Anthony Cammarato[1]

1 Department of Medicine, Division Cardiology, Johns Hopkins, Baltimore, MD

2 Department of Medicine, Division Cardiology, Northwestern University, Chicago, IL

3 Department of Biology, Illinois Institute of Technology, Chicago, IL

Sarcopenia is an age-related loss of muscle mass and function that begins in an individual's 40s, resulting in declining quality of life and mobility. However, the broad molecular impact that age has on the structure and function of muscle remains understudied, partly due to time and monetary constraints associated with long-lived mammals. Here, we present a thorough structural, molecular, and functional characterization with a detailed comparative proteomic analysis of the aging indirect flight muscle (IFM) in *Drosophila melanogaster,* a short-lived, genetically malleable model organism with a highly ordered, near 'perfect' crystalline lattice sarcomere structure. We report a progressive loss of flight between 1-, 4-, and 7-week-old flies. Further, ~30% of IFM fibers show visible damage at 7 weeks (immunofluorescence). This is paired with both sarcomere-specific and whole-cell proteomic changes (Western blot; TMT-labelled semi-quantitative mass spectrometry). K-means clustering of IFM mass spectrometry data confirm and expand upon canonical aging pathways disrupted from young to old-aged flies. Importantly, protein quality control (PQC) proteins associated with proteasomal degradation increase with age, but ubiquitin-conjugating enzymes and chaperone proteins decrease, suggesting an overall loss of PQC order and control, wherein the cell attempts to degrade proteins, but the canonical drivers of protein turnover are also reduced. Our analysis additionally confirms broad abundance decreases of translation-associated proteins, suggesting a lack of cell-signaling to support protein replacement; indeed, at 4- and 7-weeks, there is a modest increase in actin expression, and by 7-weeks of age, major cellular structural proteins such as tubulin alpha-chain 1 are decreased in expression. As these stoichiometric dysregulations could result in major structural changes, we assessed potential consequences via small-angle X-Ray diffraction on 1- and 4-week-old fly IFMs, before gross aberrations are detectable. We report a visible loss of meridional axis reflections, including a loss of intensity of ALL6, a reflection associated with the actin filament, at 4-weeks of age, suggesting a loss of thin filament order. Together, this study has generated an atlas of proteomic changes that are highly reminiscent of those found in vertebrates. Moreover, we show the consequences of genetically manipulating specific candidates that may drive age-related changes to enhance our understanding of the mechanisms by which skeletal muscle degenerates over time.

PKA PHOSPHORYLATION OF CTnI INCREASES POWER OUTPUT IN NON-PHOSPHORYLATABLE CMyBP-C PERMEABILIZED CARDIAC MYOCYTES

Laurin M. Hanft, Hannah Clevenger, and Kerry S. McDonald,
Department of Medical Pharmacology and Physiology, University of Missouri.

Our lab studies regulation of myofilament power output. We previously reported PKA increased power output in permeabilized rat cardiac myocytes (Herron et. al., *Circ Res*. 2001). Since PKA phosphorylates multiple myofilament proteins including cardiac Myosin Binding Protein-C (cMyBP-C) and cardiac Troponin I (cTnI), the molecular specificity remains uncertain. We investigated the molecular specificity of PKA regulation of power output by using a non-phosphorylatable cMyBP-C mouse model (cMyBP-C 3tSA). We tested the hypothesis that cTnI phosphorylation could augment power in non-phosphorylatable cMyBP-C myofilaments. We prepared permeabilized cardiac myocytes from cMyBP-C t3SA mice and confirmed that PKA treatment did not phosphorylate cMyBP-C but increased cTnI phosphorylation by western blot analysis. Next, cMyBP-C t3SA permeabilized cardiac myocyte preparations were attached between a force transducer and motor to measure contractile properties before and after PKA. Cardiac myocyte preparations (n = 6) averaged 139 ± 7 μm in length and 24 ± 2 μm in width. After PKA, there was a tendency for greater maximum Ca^{2+}-activated tension (before PKA 45 ± 11 $kN \cdot m^{-2}$; after PKA 48 ± 11 $kN \cdot m^{-2}$, p = 0.167) but no change in maximum Ca^{2+}-activated k_{tr} (before PKA 15.0 ± 2.7 s^{-1}; after PKA 14.8 ± 1.9 s^{-1}, p = 0.874 via paired t-test). Following PKA, force-velocity curves and power-load relationships were shifted upward during ~half-maximal Ca^{2+} activations. After PKA, peak normalized power output (PNPO) was significantly increased (before PKA 0.238 ± 0.030; after PKA 0.302 ± 0.030 $P/P_0 \cdot ML \cdot s^{-1}$, p = 0.005). We conclude that in the absence of cMyBP-C phosphorylation, PKA phosphorylation of cTnI can increase power output perhaps by causing an allosteric change in the thin filaments that reduces the propensity for cMyBP-C to interact with thin filaments.

Poster #118

Data-Driven Mechanistic Modeling of Myosin Turnover and the Effects of Mavacamten in the Cardiac Sarcomere

Brook Hodgeman[1], Colleen Kelly[2], Michael Previs[2], Wen Ma[1]

[1]Department of Physics, University of Vermont

[2]Department of Molecular Physiology and Biophysics, University of Vermont

Hypertrophic cardiomyopathy (HCM), the most prevalent genetic heart disease, is primarily driven by variants in the genes encoding myosin and myosin-binding protein C. These proteins are major components of cardiac thick filaments and play central roles in contractility. While small-molecule myosin modulators have shown clinical benefit in obstructive-HCM, heterogeneous responses underscore the need for a deeper mechanistic understanding of how these agents regulate myofilament function. Recent fluorescence recovery after photobleaching (FRAP) experiments from the Previs Lab suggest a highly dynamic equilibrium between filamentous and soluble myosin, which may be essential for thick filament maintenance and turnover. Here, we present a coarse-grained Brownian dynamics model of the cardiac sarcomere that integrates high-resolution structural data from cryogenic electron tomography (cryo-ET). The simulation captures myosin exchange kinetics through stochastic myosin dissociation, diffusion, and reassociation with the thick filament. Employing a Bayesian optimization protocol, we efficiently infer the key kinetic parameters by fitting this model to the FRAP data. Our model reproduces the measured recovery kinetics in experiments and indicates that myosin mobility in the sarcomere is governed primarily by the equilibrium between soluble and filament-bound states, rather than by hydrodynamic diffusion alone. Our analysis further suggests that the small-molecule inhibitor mavacamten slows down myosin reincorporation into the thick filament more than it impacts myosin dissociation. This computational framework provides a quantitative platform that integrates different experimental data and can be extended to dissect how various small molecules, mutations, and myosin-binding proteins regulate myofilament dynamics and function.

Increasing I-Band Titin Compliance Shifts the Structure and Function of Intact Rat EDL Muscle During Peak Twitch and Tetanic Contraction Towards a Less Active State

Anthony L. Hessel[1,2*]; Cameron Hill[3], Weikang Ma[4,5,6], Charles S. Chung[7]

[1]Institute of Physiology II, University of Muenster, Muenster, Germany
[2]Accelerated Muscle Biotechnologies, Boston, USA
[3]Randall Centre for Cell & Molecular Biophysics and British Heart Foundation Centre of Research Excellence, New Hunt's House, King's College London, London, UK
[4]Department of Biology, Illinois Institute of Technology, Chicago, Illinois, USA
[5]Center for Synchrotron Radiation Research and Instrumentation, Illinois Institute of Technology, Chicago, Illinois, USA
[6]Pritzker Institute of Biomedical Science and Engineering, Illinois Institute of Technology, Chicago, IL, USA
[7]Department of Physiology, Wayne State University, Detroit, Michigan, USA
*Corresponding Author: anthony.hessel@uni-muenster.de

Skeletal muscle contraction is not merely an all-or-nothing response but is instead regulated by various sarcomeric proteins. The titin protein is a long filament that extends across the half-sarcomere as a freely extensible viscoelastic element between the thin and thick filaments in the I-band, and runs along the thick filament backbone in the A-band. Current evidence points towards a role for titin as a regulator of contractile performance, partially determined by the viscoelastic properties of I-band titin. To explore this feature, we studied intact rat extensor digitorum longus (EDL) muscle with a spontaneous global knockout of RBM20, leading to a longer and more compliant I-band titin. Twitch and maximal tetanus contractions were produced in parallel with time-resolved (5 ms frames) small-angle X-ray diffraction at the BioCAT beamline (Advanced Photon Source) to track thick filament structures. Results indicate that homozygous muscle presents mechanical and structural signatures of impaired thick filament activation, and this is more pronounced in twitch than in tetanus. We further report on the activation-dependence of the A-band titin-based ~3.9 nm reflection (e.g., M11) that arises in the C-zone from periodic titin kinks. Titin kinks contribute to the stabilization of some myosin heads in the docked / OFF configuration and thus have a regulatory effect on these heads during contraction. We report that the titin M11 reflections undergo a noticeable change with activation, where one periodicity disappears (~3.90 nm) while another emerges (~3.97 nm), in relation to the relative activation state, suggesting an activation-dependent change in the A-band titin configuration. Regression analysis between structural markers of thick filament activation indicates that M11 reflections are highly correlated with those related to myosin head undocking from the thick filament (e.g., MLL4 intensity), indicating a close relationship between titin and docked / OFF myosin heads. Further regression analysis reveals that a more compliant I-band titin doesn't change the slope of the relationship between any pair of thick filament markers but shifts it downward, consistent with the drop in tension. Taken together, our results demonstrate that modifying I-band viscoelastic properties can alter contraction performance by regulating, but not impairing, thick filament contraction machinery.

Investigating the Physiological Role of the Interaction Between Titin Domains A168-170 and the E3 ligase MuRF1

Catherine Hoover Browne[1], Lars Wijshoff[1], Diana Bos[1], Justin Kolb[1], John Smith[1], Robbert van der Pijl[1], Henk Granzier[1]

[1]Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ

Titin is a mechanosensitive myofilament protein that spans the half sarcomere, has numerous sites for protein binding, and integrates into the thick filament. The P-zone of titin (A-band to M-band transition region) houses a binding site for E3 ligases, consisting of domains A168-A170. Multiple muscle-specific E3 ligases bind to titin domains A168-170, importantly the atrophy-associated E3 ligase MuRF1. MuRF1 has been shown to localize near the M-band in muscle cells and ubiquitinate titin at numerous sites surrounding the A168-170 binding site, including the mechanosensitive titin kinase domain (TK). It is thought that ubiquitination of the TK domain by MuRF1 induces titin degradation, particularly during muscle atrophy when MuRF1 is overactive. However, the specific role that MuRF1's binding to titin A168-170 might play in muscle physiology and the onset of muscle atrophy has not been critically studied. We sought to define the physiological role of this interaction by generating a mouse model lacking titin domains A168-170. We found this deletion to be embryonically lethal in its homozygous form, highlighting the importance of domains A168-170. As an alternative strategy to ablate MuRF1 binding without disrupting titin's domain structure, we generated a mouse model in which domains A168-170 are substituted by three non-E3 ligase binding domains ($Ttn^{A168\text{-}170\rightarrow I104\text{-}106}$). Homozygous $Ttn^{A168\text{-}170\rightarrow I104\text{-}106}$ mice have decreased viability, decreased body weights and muscle weights, and impaired force generation by the gastrocnemius complex relative to their wildtype littermates, emphasizing the critical role of domains A168-170 in muscle development and function. We tested the response of the $Ttn^{A168\text{-}170\rightarrow I104\text{-}106}$ mouse model to short-term (7 days) sciatic denervation (SD), a condition in which MuRF1 levels are highly upregulated, and found that $Ttn^{A168\text{-}170\rightarrow I104\text{-}106}$ mice responded similarly to WT mice based on muscle weights. However, disruptions to sarcomere structure following SD are more prominent in $Ttn^{A168\text{-}170\rightarrow I104\text{-}106}$ mice than WT mice. These preliminary findings are consistent with the hypothesis that MuRF1 binding and ubiquitination of titin's A168-170 segment is an important regulator of muscle size and function.

Investigation of the Effects of Troponin I Acetylation on Thin Filament Activation with Molecular Dynamics

Vinay P. Jani[1,2], Kristina B. Kooiker[1], Anthony Asencio[2], An-Yue Tu[2],

Matthew Childers[1], Michael Regnier[2], Farid Moussavi-Harami[1]

[1]Division of Cardiology, [2]Department of Bioengineering, University of Washington, Seattle, WA

Acetylation is one class of PTMs of recent interest as pharmacologic inhibition of deacetylases have been identified as a recent therapeutic target of target of interest. Most studies have used acetyl-mimetics for in vitro investigation, as in the case of the K131 acetylation site on cTnI[1], though for many residues, the mechanism by which acetylation alters sarcomere function is unknown. Here, we utilized molecular dynamics simulations to study cardiac troponin I (cTnI) acetylation on thin filament activation. Simulations were performed for at least 100 ns using the cardiac troponin complex (PDB 1J1E) with Mg^{2+} in sites III/IV and Ca^{2+} in site II of cardiac troponin C (cTnC). Troponin residues that are known to interact with actin and tropomyosin were then constrained. We then acetylated *in silico* all reported cardiac troponin I (cTnI) residues that have been identified to be acetylated, K72, K106, K117, K131, K174, K177, and K178 and compared the dynamics of the cTnI switch peptide (resides 148-163) and the cTnC hydrophobic patch (residues 44, 45, 52, 56, and 60). We find that the root means square distance (RMSD) of both the cTnI switch peptide and cTnC hydrophobic patch is increased in acetylated vs. WT simulations, while the distances between the coordinating oxygen and calcium were similar. This would suggest that the switch peptide and hydrophobic patch exhibit more flexibility with acetylation. To determine whether this reflected instability of the troponin ON state as is often the case, we quantified the radium of gyration and surprisingly found it to be reduced with acetylation. Moreover, more contacts between the hydrophobic patch and switch peptide were identified with acetylation. These results show that despite the increase in flexibility, there is an increase in the number of interactions between the switch peptide and hydrophobic patch, suggesting that cTnI acetylation may help to promote the thin filament ON state and actin binding. This finding was supported experimentally with fluoroscopy as treatment of cTnI with acetic anhydride enhanced binding between cTnI and cTnC. Current studies are now underway to determine the effect of each acetylated residue on cTnI to identify potential therapeutic targets.

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A Mechanistic Cross-Bridge Model of Cardiac Muscle Mechanics Under Energetic Stress

Filip Jezek[1], Anthony Baker[2], Dan Beard[1]
1 University of Michigan Medical School, Ann Arbor, MI, USA,
2 San Francisco VA Health Care System and Department of Medicine, San Francisco, CA, USA

Most forms of heart failure are accompanied by energetic deficit — impaired mitochondrial ATP production, elevated free ADP, and decreased PCr/ATP ratio — that compromises myosin cross-bridge cycling. Computational models linking mitochondrial metabolism to sarcomere mechanics [1] offer a path to understanding this coupling. A strain-discretized cross-bridge model by Beard et al. [2] applied on distinct ATP force-velocity data does not reproduce ATP-dependent contractile differences. To address this, we extend the model by including additional physical mechanisms and furthermore test the model with new data sets that include slack-restretch protocols across ATP levels.

Experiments on mouse right-ventricular cardiac trabeculae provide force-velocity and slack-restretch data at 8 mM (saturating), 2 mM (compromised), and 0.2 mM (depleted) ATP. Model calibration targets discrete features, including unloaded shortening velocity (Vmax), isometric force (F0), force-velocity profile, force redevelopment rate (ktr) at multiple sarcomere lengths, peak restretch force, viscoelastic force peak, strain-related detachment signatures and others. Feature analysis across ATP levels demonstrates systematic differences in contractile behavior, while the multi-condition dataset helps discriminate between mechanistic hypotheses.

We present a six-state strain-discretized probabilistic model. States comprise two disordered-relaxed (DRX) pools by nucleotide occupancy (ATP and ADP·Pi), two strain-sensitive attached states (pre- and post-power-stroke), and two super-relaxed (SRX) pools by nucleotide state. This explicit representation of ATP catabolism intermediates lets [ATP] and [ADP] modulate transition rates biochemically throughout the cycle. Asymmetric strain-dependent rate modifiers on the attached states capture velocity dependence of the power stroke and detachment. Force-dependent SRX mobilization encodes thick filament mechanosensing and titin passive mechanics incorporates Ca^{2+}-dependent viscoelasticity [3].

The extended model qualitatively reproduces all key features of both force-velocity and slack-restretch protocols, establishing a framework capable of simulating contractile behavior across conditions. This provides the necessary foundation for mechanistic identification of ATP-dependent contractile differences — the immediate next step — and for future coupling to mitochondrial ATP production models [1] to evaluate metabolic interventions in heart failure.

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Phase separation controls cardiac myosin light chain kinase activity

Kyler C. Carl[1], Ivanka R. Sevrieva[1], Austin Welette-Hunsucker[1], Kenneth S. Campbell[1], Xu Fu[2], Elisabeth Ehler[3], Thomas Kampourakis[1]

[1]Division of Cardiovascular Medicine, College of Medicine, University of Kentucky, Kentucky, Lexington, USA

[2]Light Microscopy Core, College of Medicine, University of Kentucky, Kentucky, Lexington, USA

[3]Randall Centre for Cell and Molecular Biophysics, King's College London, London, SE1 1UL, United Kingdom; and King's College London British Heart Foundation Centre of Research Excellence, Guy's Campus, SE1 1UL, United Kingdom

Interdigitating actin-containing thin and myosin-containing thick filaments comprise the smallest contractile unit of striated muscle, the sarcomere. In addition to the well-studied classical Ca^{2+}-dependent thin filament regulatory pathway of muscle contraction, activation of the thick filaments themselves has emerged as a second regulatory step that controls cardiac myofilament contractile function. Similar to the thin filaments, thick filaments are believed to exist in both a diastolic OFF and systolic ON state, and the rate of transition between those states are likely rate-limiting for systolic force development and diastolic relaxation of the heart. Understanding the mechanisms underlying the regulatory transition of the thick filaments is crucial for understanding the pathogenesis of cardiac disease and heart failure, as well as for identifying viable targets for therapeutic intervention.

Phosphorylation of the myosin regulatory light chain is an important regulatory mechanism in the heart that controls cardiac output and performance by modulating both myosin motor availability and activity. Myosin motors are frequently found hyperphosphorylated in human myocardium from patients with heart disease and failure. The primary enzyme responsible for phosphorylating the myosin heads in the heart is the cardiac isoform of myosin light chain kinase (cMLCK; MYLK3), which shows unique cardiac-specific features, distinguishing it from both the skeletal and smooth muscle/non-muscle isoforms. However, in contrast to its homologues, the regulatory mechanisms underlying cMLCK localization and function have remained elusive.

In this study, we provide mechanistic evidence that cMLCK is fine-tuned to operate in the environment of biomolecular condensates, which is fundamentally different to the common concept of kinases signaling via in-situ phosphorylation of protein targets. We show that cMLCK spontaneously undergoes liquid-to-liquid phase separation controlled by its unique N-terminal extension, resulting in the formation of biomolecular condensates that localize to the sarcoplasmic reticulum in human cardiomyocytes. cMLCK condensates can actively recruit both co-factors and substrates, controlling both its activity and specificity. Collectively, our study has uncovered an underappreciated mechanism for myofilament contractile regulation

BAG3 interacts with RNA binding proteins to mediate protein turnover in the sarcomere

Lucy Plenge, Marcus Wagner, Jaime Yob, Graham Branscom, Michael Morley, Sharlene Day

The Division of Cardiovascular Medicine and Cardiovascular Institute, Perelman School of Medicine

Hypertrophic Cardiomyopathy (HCM) is an inherited heart condition characterized by increased left ventricular mass. Although pathogenic variants in genes coding for sarcomeric proteins have been identified, there is further need to understand the genetic drivers of HCM. Genomic studies have identified common variants in or near *BAG3* as risk alleles for HCM. BAG3 is important for maintaining cellular proteostasis, but its role in HCM pathophysiology is unknown. To define potential client proteins of BAG3, we performed biotin proximity labeling by expressing the BAG3-TURBO fusion protein via adenoviral transduction in iPSC-derived cardiomyocytes (iPSC-CMs). By gene ontology analysis, myofilament proteins made up the greatest proportion of proteins proximal to BAG3. RNA-binding proteins (RBPs) were the second most abundant category of proteins proximal to BAG3, comprising 17% of its interactome. This finding led us to perform shRNA-mediated knockdown of BAG3 in iPSC-CMs followed by RNA sequencing to determine the impact of BAG3 on the cardiomyocyte transcriptome. Transcript expression of many sarcomere genes were significantly decreased upon BAG3 knockdown. Western blots after BAG3 knockdown showed discrepancies between gene and protein expression with many sarcomere proteins being similarly abundant upon BAG3 knockdown despite decreased transcript expression. We hypothesized that BAG3 may regulate sarcomere gene translation indirectly by stabilizing RBPs. To investigate this hypothesis, we performed TURBO-ID biotin proximity labelling followed by RNA immunoprecipitation sequencing (RIP-seq) in iPSC-CMs to identify mRNA transcripts proximal to BAG3. Gene enrichment analysis compared the abundance of transcripts proximal to BAG3 to their abundance in iPSC-CM lysate to identify enriched and depleted genes proximal to BAG3. We observed that mitochondrial transcripts proximal to BAG3 were relatively depleted while genes involved in signaling, transcription regulation and sarcomere structure were enriched. For a number of transcripts, there was a direct concordance between BAG3 proximity enrichment and downregulation after BAG3 knockdown. Local translation of sarcomere proteins is important for cardiac sarcomere functioning, and BAG3 localizes strongly to the cardiac Z-disc. Our results to date support the hypothesis that BAG3 stabilizes RNA-binding proteins, indirectly regulating the translation of transcripts of sarcomere genes either through stabilization, localization, or assembly of the translation complex

Poster #125

A mechanism for how unstructured regions in the myofilament protein ABLIM1 impact cardiac contractility

Bin Sun[1], Alec Loftus[2], Peter Kekenes-Huskey[2]

Harbin University[1], Loyola University Chicago[2]

The heart adapts to shifting mechanical demands through the precise, regulated chemical modification of its contractile proteins. Many of these changes, such as phosphorylation, occur within the intrinsically disordered regions (IDRs) of proteins that compose the myofilament. While IDRs have historically been challenging to characterize relative to their folded counterparts, the discovery of flexible IDR segments acting as dynamic, tunable regulators of protein behavior continues to accelerate, motivating a need to understand corresponding mechanisms at the molecular level. Furthermore, because cardiac dysfunction often coincides with post-translational modification (PTM) patterns distinct from those observed in healthy hearts, mapping how IDR alterations directly impact the sarcomere is a critical step in understanding heart failure.We hypothesized that phosphorylation regulates the actin-binding protein ABLIM1 by reshaping its conformational ensemble and modulating its interactions with other nearby myofilament proteins. To test this, we employed a multiscale modeling approach to predict ABLIM1 behavior under conditions of reduced phosphorylation resulting from diminished GSK3β activity. By relating ABLIM1's IDR properties to the effective concentrations of its C-terminal binding regions adjacent to neighboring actin filaments, we show that localized physicochemical changes from phosphorylation significantly alter ABLIM1 IDR ensemble properties, leading to allosteric impacts on its C-terminal binding interactions and, consequently, force generation in the sarcomere. Finally, we summarize our progress in reassembling complete ABLIM1 structures from independent simulations of IDR segments and ensuring self-consistency across the multiscale framework via shared "figures of merit." We intend for this framework to provide a mechanistic basis for bridging molecular-level changes in myofilament-associated proteins with intrinsic disorder (MAPIDs) to macroscopic biomechanical responses.

Neutrophils Drive Myocardial Dysfunction in Takotsubo Syndrome

Elias Rawish[1,2,3], Katharina Kurz[1,2,3], Carolin Gragoll[1,2,3], Akhil Baby[10], Pascal Bouvain[4], Ivana Androšević[5], Jorge Duque Escobar[2,3,6], Timo Rusack[1,2,3], Thomas Pühler[2,3,7], Timo Gemoll[8], Markus Hoffmann[5], Tanja Zeller[2,3,6], Sakthivel Sadayappan[10], Walter Raasch[9], Thomas Kurz[1,2,3], Ulrich Flögel[4], Ingo Eitel[1,2,3], Thomas Stiermaier[1,2,3]

1 Medical Clinic II, University Hospital Schleswig-Holstein, Lübeck, Germany
2 DZHK (German Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Germany
3 University Heart Center Lübeck, Lübeck, Germany
4 Experimental Cardiovascular Imaging, Institute for Molecular Cardiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
5 Institute for Systemic Inflammation Research (ISEF), University of Lübeck, Lübeck, Germany
6 Institute for Cardiogenetics, University of Lübeck, Lübeck, Germany
7 Clinic of Cardiac and Thoracic Vascular Surgery, University Hospital Schleswig-Holstein, Lübeck, Germany
8 Section for Translational Surgical Oncology and Biobanking, Department of Surgery, University of Lübeck and University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany
9 Institute for Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany
10 Department of Cellular and Molecular Medicine, University of Arizona, College of Medicine, Tucson, AZ, USA

Takotsubo syndrome (TTS) is characterized by acute left ventricular systolic dysfunction that, despite its usually transient nature, confers a substantial risk of morbidity and mortality comparable to that of acute myocardial infarction (MI). Typically affecting post-menopausal women following a period of intense stress, TTS presents with clinical features that mimic MI, yet without culprit coronary artery disease. Current management is largely supportive in the absence of dedicated treatment approaches. However, mechanism-targeted therapies are urgently needed to improve acute outcome and prevent or mitigate persisting symptoms. We integrated real-world data, plasma proteomics, longitudinal deep immunophenotyping, myocardial tissue analyses, and an epinephrine-induced murine TTS model with neutrophil-specific ^{19}F cardiac magnetic resonance imaging to define the role of neutrophils in TTS. In patients, admission neutrophilia correlated with left ventricular dysfunction and higher mortality. Proteomics revealed upregulation of neutrophil-attracting and endothelial-activating mediators, and activated neutrophils accumulated in myocardial tissue. In vivo, neutrophil-specific ^{19}F signal colocalized spatiotemporally with dysfunctional ventricular segments and regressed during functional recovery. Mechanistically, catecholamine stress mobilized mature neutrophils from bone marrow, while endothelial P- and E-selectins mediated myocardial recruitment; selectin blockade reduced infiltration and preserved systolic function. Downstream, neutrophil myeloperoxidase drove oxidative injury, whereas colchicine reduced neutrophil activation and trafficking, lowered myocardial reactive oxygen species production, improved mitochondrial function, and increased ejection fraction. These findings identify neutrophil-derived oxidative stress as a central, therapeutically targetable driver of myocardial dysfunction in TTS.

Poster #127

Dynamic Allosteric Regulation of Myosin by Cardiomyopathy Mutations and Small-Molecule Modulators

Wen Ma[1,2]

[1] *Department of Physics, University of Vermont*

[2] *Cellular, Molecular, and Biomedical Sciences Program, University of Vermont*

Myosin is a sophisticated allosteric machine whose function is sensitive to local perturbations induced by point mutations and ligand binding. In particular, mutations in myosin can disrupt force generation and lead to cardiomyopathies that impair cardiac pump function. Recent high-resolution structural studies revealed snapshots of an autoinhibited myosin state whose misregulation is strongly associated with cardiomyopathies. An important open question is what determines whether a mutation primarily affects the intrinsic motor properties or the stability of the autoinhibited state of myosin. In addition, it remains unclear why myosin inhibitors and activators, despite binding to the same allosteric site, exert completely opposite effects on myosin function. Here, we present a computational framework that integrates AlphaFold-based modeling with enhanced-sampling molecular dynamics simulations to investigate how mutations and ligands regulate myosin motor activity and activation. We show at the atomistic level that a dilated cardiomyopathy mutation (E525K), despite promoting some aspects of the intrinsic motor transition, inhibits activation of the myosin double heads. Our approach allows direct characterization of the regulatory effects of a small-molecule inhibitor and an activator on both the isolated motor domain and the autoinhibited state. Our simulations identify the key allosteric residues that transmit ligand-induced signals along the motor activation pathway, revealing the conformational dynamics that encode allosteric communication. The atomistic insights gained here enable the design of novel myosin modulators to counteract the effects of cardiomyopathy mutations.

Troponin Enhanceropathies: A Novel Role for the Troponin Genes

Jenna Thuma[1], Yvette Wang[2], Madhoolika Bisht[2], Jordan King[2], Amelia Van Aelst[2], Erin Blevins[3], Svetlana Tikunova[2], Marco Coutinho da Silva[3], Christoph Lepper[2], Brandon Biesiadecki[2], Jonathan Davis[1,2]

1. Biophysics Department, Ohio State University, Columbus, OH
2. Department of Physiology and Cell Biology, Ohio State University, Columbus, OH
3. Theriogeneology and Reproductive Medicine, Ohio State University, Columbus, OH

Striated muscle contraction is regulated by the troponin complex which consists of three proteins: troponin C (TnC), troponin I (TnI), and troponin T (TnT). Cardiac troponins' protein role in the heart is well known and documented; however, we are proposing a novel role for the cardiac troponins at the DNA level. DNA regulatory elements are important regions that control cell and time specific gene expression. These types of elements are generally found intergenically or intronically, but rarely they can also be found within exons. Shockingly, we found evidence that all three cardiac troponin genes contain these exonic DNA regulatory elements. Thus, any nucleotide mutation in their exonic DNA regulatory element would not only change protein structure and function in the heart, but also the function of the regulatory element. This could result in altered expression of the genes that the element controls. Although we believe this occurs in all three subunits, here we focus on the inhibitory subunit, TnI (Tnni3). First, we confirmed regulatory activity by luciferase assays then used chromosome conformation capture databases to determine the regulatory targets of this element. The regulatory elements within cardiac TnI appear to interact with three genes, slow skeletal TnT (Tnnt1), Rdh13, and Dnaaf3. TnI knockout mice have diaphragm weakness consistent with TnT dysregulation, whereas we observe both gross internal organ and sperm abnormalities consistent with Dnaaf3 and Rdh13 dysregulation. Furthermore, in a KI model (only different by 4nt), we see the same abnormal anatomy and corresponding differential expression of Tnnt1 in skeletal muscles as well as Dnaaf3 and Rdh13 in the reproductive organs by qPCR. **Thus, we are proposing a novel DNA regulatory role for the cardiac troponins, independent of their protein function, that, when mutated, may cause non-cardiac disease.**

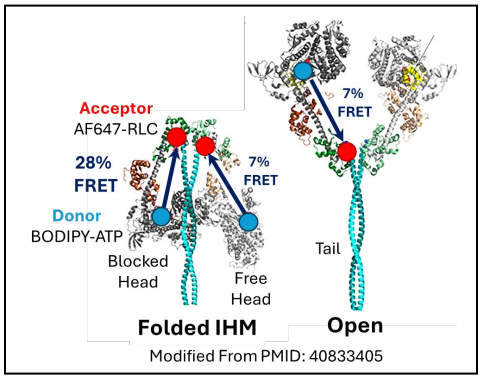
Seeing is believing at the single molecule level: Are myosin's IHM and SRX states inextricably linked?

Shane R Nelson[1], Jinghua Ge[2], Rama Reddy Goluguri[3], Kathleen M Ruppel[3], James A Spudich[3], Christopher M Yengo[2], David M Warshaw[1]

[1]Department of Molecular Physiology & Biophysics; Cardiovascular Research Institute, University of Vermont, Burlington, VT 05405

[2]Department of Cell and Biological Systems, Penn State College of Medicine, Hershey, PA 17033
[3] Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

The myosin super-relaxed (SRX) state is an autoinhibited, energy-conserving state that regulates muscle energetics and contractility. It is biochemically defined by an extremely slow ATPase rate (~0.005 s^{-1}) and may correspond to the interacting heads motif (IHM), though the relationship between these biochemical and structural states remains debated. To address this issue, we simultaneously measured the structural and biochemical states of individual recombinant human β-cardiac myosin HMM molecules adhered to a glass surface using single-molecule fluorescence techniques.



Myosin's biochemical states (SRX vs DRX) were determined from the lifetimes of the observed turnover of single molecules of fluorescent BODIPY-ATP. Structural information was simultaneously obtained using single-molecule Förster Resonance Energy Transfer (FRET) between BODIPY-ATP (donor) in myosin's active site and an AlexaFluor647 (acceptor) labeled regulatory light chain (RLC). In the IHM structure, the "blocked head" configuration brings the two fluorescent probes within ~7nm of each other, enabling high FRET efficiency, whereas other myosin configurations (e.g., IHM "free" head and "open" conformations) increase probe separation (~9nm) resulting in lower FRET efficiency.

The lifetime distributions of ATP turnover events were best described by two exponentials ($p < 0.001$ vs. single-exponential, likelihood ratio test) with time constants (reciprocal rates) consistent with literature values for the DRX and SRX states (65s and 192s, respectively). Apparent FRET efficiencies from these same ATP turnover events were in close agreement with predicted values (see figure), defined by two populations: a low-FRET population (8±5% efficiency, 70% of events) and high-FRET population (25±15% efficiency, 30% of events). High-FRET events exhibited significantly longer lifetimes than low-FRET events ($p=0.002$). Comparisons to Monte Carlo simulations indicate that high-FRET events correspond almost exclusively with SRX kinetics, suggesting tight coupling between the SRX and IHM states. These studies will be extended to include conditions known to perturb the SRX and IHM states such as salt, myosin mutations, and small molecule inhibitors and activators.

The Sweet Taste Receptor Regulates Cardiomyocyte Contractility and May Regulate Cardiomyocyte Bioenergetics

Authors: Yoder MW, Papadaki M, Kelly ED, Zied AGA, Markle B, Minh D, Muller GK, Kirk JA

GPCR-based taste receptors are expressed in taste buds where they mediate the perception of bitter, umami, and sweet taste qualities. Sweetness is detected by a heterodimer of type I taste GPCRs (TAS1R2/TAS1R3). Recently, extra-oral sweet taste receptors (STRs) were found in tissues such as the airways, brain, pancreas, and skeletal muscle where they are proposed to function nutrient sensors. Previous literature has shown that cardiomyocytes express TAS1R mRNA, including tas1r1 and tas1r3 transcripts. However no study has followed up to investigate tas1r2 expression, protein-level expression of the TAS1R family, nor functional implications of cardiomyocyte STR signaling.

Here, we show that both TAS1R2 and TAS1R3 subunits comprising the STR are expressed in human and mouse myocardia, localized to the plasma membrane. We demonstrate in adult mouse cardiomyocytes and iPSC-derived engineered heart tissue that STR stimulation with aspartame rapidly modulates excitation-contraction coupling, quickening calcium handling and increasing contractility. To investigate how STR agonism influences cellular signaling, we activated neonatal rat ventricular cardiomyocyte STRs and evaluated spatial proteomic and whole-cell proteomic changes by mass spectrometry. Our data revealed localization changes to kinases such as CAMKIID, SPEG, and PKC. Correspondingly, we investigated phosphorylation changes upon STR stimulation, revealing PTM consequences to many myofilament and EC coupling regulatory proteins. Moreover, we show mitochondrial proteome alterations, including the loss of membrane-enriched electron transport chain components as well as regulators of mitochondrial fission and fusion. One significantly membrane-enriched protein was MAP1LC3A. MAP1LC3A canonically accumulates on membranes to facilitate autophagy and mitophagy activation. To investigate whether STR signaling may regulate mitochondrial health, we performed live-cell imaging using both mitochondrial and lysosomal dyes. Aspartame treatment here induced a higher degree of colocalization between the two organelles, suggesting a possible link between nutrient sensation and cardiomyocyte bioenergetics.

Previous studies have shown that high sugar diets increase the risk of arrhythmia. We hypothesized that this pathogenic phenotype may result from a direct overstimulation of the cardiac STR. We prolonged aspartame treatment in isolated adult mouse cardiomyocytes, revealing an increased frequency of arrhythmic beats. Further, we also show that TAS1R2 is upregulated in human heart failure. Adenoviral-mediated overexpression of TAS1R2 in engineered heart tissues resulted in arrhythmic beating pattern when exposed to aspartame.

Together, these results begin to uncover a novel GPCR pathway altering cardiac physiology and energetics in response to extracellular nutrient detection. Moreover, we provide evidence of pathophysiological relevance to their signaling thus representing a potential therapeutic axis in heart failure and arrhythmia.

Poster #131

Desensitization of the Cardiac Troponin Complex by TnI Phosphorylation and Epigallocatechin-3-Gallate

Helene Tigro[1] and Christopher Solis[1]*

[1]Department of Health, Nutrition, and Food Sciences, Florida State University, Tallahassee, FL 32306

*Corresponding author: csolis@fsu.edu

The objective of this model is to investigate the mechanisms behind desensitization of thin filaments to Ca^{2+} due to troponin I (TnI) Ser23/24 phosphorylation and (-)-epigallocatechin-3-gallate (EGCG) binding. The hypothesis is that TnI phosphorylation and EGCG binding desensitize thin filaments via enhanced stabilization of protein-protein interactions within the troponin complex. AlphaFold 3 recreated *de novo* structural models of phosphorylated TnI bound to the troponin (Tn) complex. Molecular docking simulations modeled EGCG binding to Tn. AlphaFold 3 revealed that TnI phosphorylation increased the distancing of the TnI N-terminus from the TnC N-lobe due to the formation of an α-helix at the Ser23/24 phosphorylation sites. Molecular docking simulations localized the EGCG binding site in the Tn complex that resulted from hydrogen-bonds between EGCG and the troponin C (TnC) C-lobe (residues 120-161) as well as at the beginning of the TnI IT arm and the TnI N-terminus where Ser 23/24 phosphorylation sites lie. The results suggest that EGCG and TnI Ser23/24 phosphorylation desensitization mechanisms are potentially allosteric, with phosphorylation modifying interactions between TnI and TnC N-terminal domains and EGCG between TnI and TnC C-terminal domains. The results of this work could inform the development of more targeted therapies to treat diastolic heart diseases such as HFpEF and cardiomyopathies derived from over-sensitization of thin filaments to Ca^{2+}.

Disease-associated MYH11 tail variants disrupt smooth muscle myosin II filament assembly and contractility in vascular smooth muscle cells

Authors: Sasha K.Demeulenaere, Margaret A. Bennet, Bradley Sommefield, Hiral Patel, Gregory Aubert, Dianna Milewicz, Patrick W. Oakes, Jordan R. Beach

Abstract:
Smooth muscle myosin II (SMII) is the principal motor protein that powers vascular smooth muscle contraction and supports arterial wall mechanics. Rare variants in MYH11 are linked to inherited aortic disease, including aneurysms and dissections, highlighting the importance of the myofilament in vascular homeostasis. To define SMII behavior in a physiologic context, we developed a CRISPR knock-in EGFP-SMII mouse model, enabling direct measurement of SMII organization and dynamics in primary vascular smooth muscle cells (VSMCs) and whole arterioles. Using this model, together with cultured VSMCs, we found that SMII is largely filament-associated yet highly dynamic at baseline, and becomes further assembled and acutely stabilized during agonist-induced contraction. This framework suggests that MYH11-associated aortic disease may arise not only from altered motor output but also from disruption of SMII filament assembly and stabilization.

We therefore set out to investigate two disease-linked SMII tail variants, A1839V, a variant of uncertain significance discovered in a Loyola University patient, and K1256del, a known pathogenic mutation, to determine how they alter SMII organization and whole-cell mechanics. EGFP-tagged WT and variant SMII were expressed in human VSMCs, and single-cell permeabilization assays were used to quantify filament assembly, with complementary live-cell imaging, FRAP, and traction force microscopy to assess filament organization, exchange dynamics, and whole-cell contractility.

Both variants reduced the assembled SMII fraction relative to WT and increased SMII exchange kinetics. Both also disrupted normal myosin-rich stress fiber architecture, with A1839V producing shortened, disorganized fibers and K1256del producing more severe sheet-like networks and cytoplasmic aggregates. Live imaging revealed that K1256del filaments are especially unstable relative to WT and A1839V. Functionally, both variants impair force generation in VSMCs, and preliminary data indicate that K1256del also decreases focal adhesion number and maturation.

Together, these findings support a model in which MYH11 tail variants destabilize SMII filament assembly and exchange, impair actomyosin organization, and weaken force production in VSMCs. We hypothesize that this loss of myofilament stability not only compromises acute contractile function but may also drive VSMC dedifferentiation away from a differentiated contractile state by disrupting the cytoskeletal and adhesion-based mechanical cues that help maintain smooth muscle identity. This work suggests that defective SMII assembly and stabilization represent a candidate mechanism linking disease-associated MYH11 variants to both altered VSMC mechanics and phenotypic modulation in inherited aortic disease.

SARCOMERE LENGTH-DEPENDENCE OF THE STRUCTURAL CHANGES IN THE THICK FILAMENT OF DEMEMBRANATED MYOCARDIAL SLICES FROM THE RABBIT HEART

Watson G.[1,2], Arcidiacono A.[1,2], Kalakoutis M.[1,2], Hill C.[1,2], Shattock MJ.[2,3], Clark JE.[2,3], Theyencheri N.[4], Irving M.[1,2], Fusi L.[5], Brunello E.[1,2]

Affiliations:

[1] Randall Centre for Cell and Molecular Biophysics, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.
[2]British Heart Foundation Centre of Research Excellence, King's College London, London, United Kingdom.
[3]School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, United Kingdom.
[4]ESRF – The European Synchrotron Radiation Facility, Grenoble, France.
[5]Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, King's College London, London, United Kingdom.

Contraction of the myocardium is regulated at the myofilament level. Calcium-dependent structural changes in the thin filaments control access to myosin binding sites on actin, while thick filament-based regulation controls the number of available myosin motors. Motors are folded and helically ordered on the thick filament surface in diastolic cardiac muscle, and become more perpendicular to the filament backbone during activation. Increasing sarcomere length (SL) increases the calcium sensitivity of force, a phenomenon termed length-dependent activation (LDA), however the underlying mechanisms remain unclear. Here we used Small-Angle X-ray Diffraction (SAXD) at beamline ID02 (ESRF, France) to measure the SL-dependence of the structural changes in thick filaments of demembranated myocardial slices from the left ventricular wall of cardioplegically arrested rabbit hearts. Myocardial slices were activated at calcium concentrations ranging from pCa 9 to pCa 4.7 in the presence of 3% Dextran T500, from short (2.05 μm) or long (2.25 μm) starting SL with a temperature jump to 37°C and in SL-isometric conditions during force development. Active force had sigmoidal dependence on calcium concentration and was approximately doubled at long SL during maximal activation. Correspondingly, at long SL there was greater calcium-dependent increase in the equatorial intensity ratio ($I_{1,1}/I_{1,0}$), associated with movement of myosin motors from the thick filaments towards the thin filaments. Furthermore, a larger decrease in the intensity of the first myosin layer line (I_{ML1}), and larger increase in the intensity of the low-angle peak of the M3 reflection (L_{M3}), signaled greater departure of myosin motors from their helical order and an increase in the number of perpendicular force-generating motors at long SL. Increased SL potentiated structural changes in the myosin filaments at systolic but not diastolic calcium levels, demonstrating that LDA requires both stretch and calcium. Inhibition of myosin with Mavacamten abolished active force and the associated structural changes in the thick filaments. Our results elucidate stretch- and calcium-dependent regulatory structural changes in the myosin filament in cardiomyocytes of the ventricular wall, which underpin the regulation of ventricular contractility, and demonstrate the suitability of myocardial slices for SAXD studies of myofilament regulation in large mammal hearts.

Deep Learning as a Surrogate Myofilament Model: Predictive AI for Cardiac Sarcomere Dynamics

Yasser Aboelkassem[1,2]
[1]Engineering and Technology, University of Michigan-Flint
[2]Michigan Institute for Data and AI in Society, University of Michigan

Cardiac contraction emerges from tightly coupled, nonlinear interactions among calcium activation, cross-bridge cycling, and sarcomere mechanics. While mechanistic myofilament models provide critical biophysical insight, they are often computationally expensive and limited in their ability to generalize across protocols, parameter regimes, and species. Here, we introduce a deep learning model in which artificial neural networks (ANNs) serve as accurate, biophysically consistent surrogates for cardiac sarcomere dynamics. Trained on synthetic datasets generated from established myofilament models across isosarcometric, isometric, isotonic, and cell-shortening protocols, the ANN learns the intrinsic nonlinear relationships among calcium concentration, sarcomere length, stiffness, temperature, and force production. Systematic analysis of network depth shows that shallow ANN architectures underfit and fail to reproduce transient twitch dynamics, whereas optimized deep networks achieve orders-of-magnitude error reduction, stable convergence, and strong generalization. The trained models accurately reproduce hallmark physiological behaviors, including steady-state force-Ca^{2+} relations, force-velocity curves, and time-resolved shortening and force transients. This work demonstrates that AI is not merely a post-processing tool but can function as the model itself, learning the governing dynamics of myofilament behavior. By bridging mechanistic modeling and data-driven learning, this approach enables rapid, scalable, and predictive simulations of sarcomere function, opening new avenues for leveraging experimental datasets and for modeling cardiac disease and remodeling at the myofilament level.