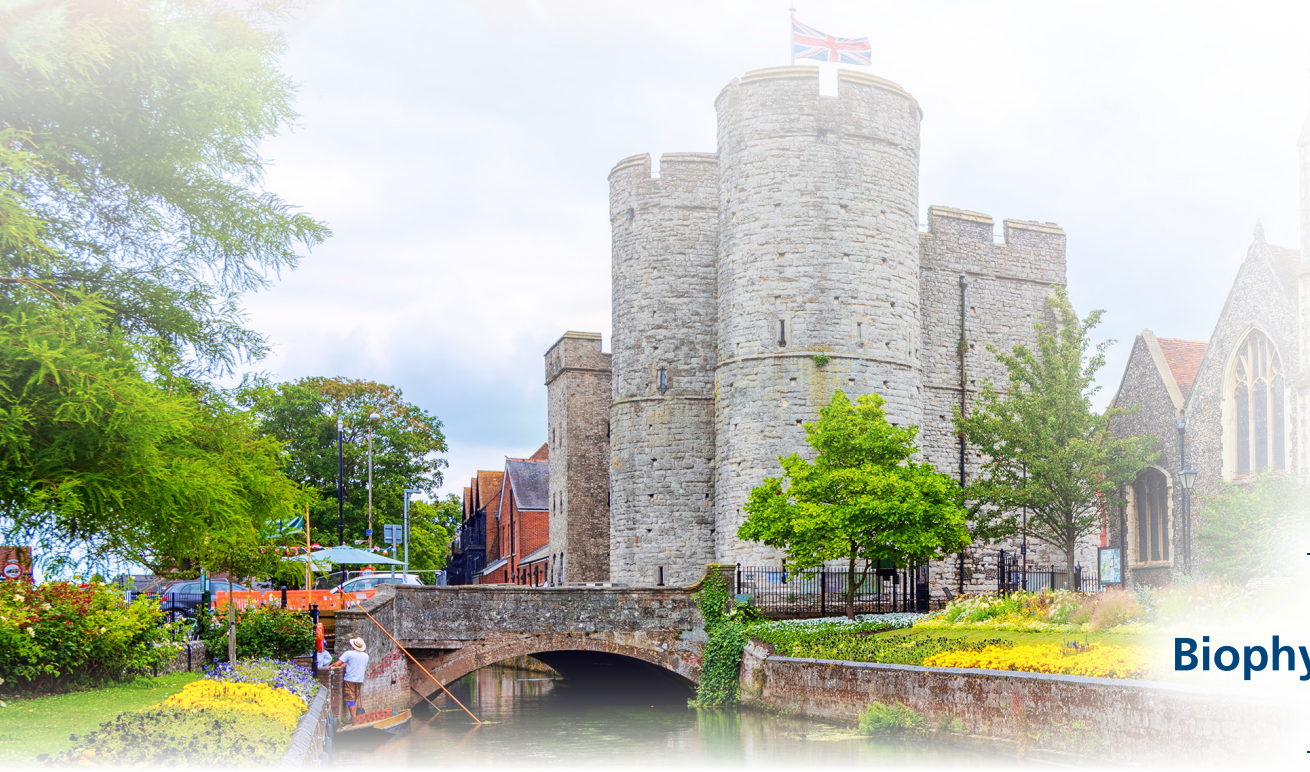
Towards a More Perfect Union: Multi-Scale Models of Muscle and Their Experimental Validation

Canterbury, England | July 17–20, 2023



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Biophysical Journal

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July 2023

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting *Towards a More Perfect Union: Multi-Scale Models of Muscle and Their Experimental Validation*, co-sponsored by the University of Washington Center for Translational Muscle Research. The thematic meeting series provides an opportunity for scientists in a more focused research area to meet and exchange ideas in different locations around the world in an environment that is more intimate than large annual meetings. We hope that this will provide a venue that stimulates discussion about recent exciting new advances in knowledge of muscle structure-function at multiple scales and how a new generation of technological advances in experimentation and computational approaches can be leveraged to provide new insights into the mechanisms of contraction and its dysfunction with muscle diseases.

We are living through a period of rapid technological advances in gene engineering, molecular and cellular biology, and high-resolution imaging. For example, a revolution in cryo-electron microscopy and image reconstruction together with the enhanced power of computational modeling is beginning to provide high-resolution structures that offer mechanistic insight and, with sufficient validation, will become predictive tools to study the effects of disease-causing mutations. The new generation of experimental and computational modeling approaches that is emerging can also span multiple scales and provide both temporal and spatially-explicit properties and information.

We hope that you will all actively take part in the discussions following each talk, in the poster sessions, and in the informal exchanges that will be possible during the coffee breaks, lunches, and the banquet. We also hope that you will enjoy the historic and beautiful surroundings of Canterbury and the local area!

The Organizing Committee

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Biophysical Society Code of Conduct, Anti-Harassment Policy

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all, regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit.

All BPS meetings and BPS-sponsored activities promote an environment that is free of inappropriate behavior and harassment by or toward all attendees and participants of Society events, including speakers, organizers, students, guests, media, exhibitors, staff, vendors, and other suppliers. BPS expects anyone associated with an official BPS-sponsored event to respect the rules and policies of the Society, the venue, the hotels, and the city.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Attendees or participants who are asked to stop engaging in harassing behavior are expected to comply immediately. Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed is NOT REQUIRED to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if the complainant does not feel comfortable with such an approach, they can report the behavior as detailed below.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Reporting a Violation
Violations of this Conduct Policy should be reported immediately. If you feel physically unsafe or believe a crime has been committed, you should report it to the police immediately.

To report a violation to BPS:

- You may do so in person at the Annual Meeting at the BPS Business Office in the convention center.

- You may do so in person to BPS senior staff at Thematic Meetings, BPS Conferences, or other BPS events.

- At any time (during or after an event), you can make a report through http://biophysics.ethicspoint.com or via a dedicated hotline (phone numbers listed on the website) which will collect and relay information in a secure and sensitive manner.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated per the procedure detailed below. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Investigative Procedure

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised, nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.

Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation. Please note, if a complaint is filed anonymously, BPS may be severely limited in our ability to follow-up on the allegation.

- An impartial investigative committee, consisting of the current President, President-Elect, and Executive Officer will be established. If any of these individuals were to be named in an allegation, they would be excluded from the committee.

- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.

- The committee will speak to the alleged offender and present the complaint.

- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.

- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.

- The investigative committee may seek BPS Counsel's advice.

- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

- If the severity of the allegation is high, is a possible repeat offense, or is determined to be beyond BPS's capacity to assess claims and views on either side, BPS may refer the case to the alleged offender's home institution (Office of Research Integrity of similar), employer, licensing board, or law enforcement for their investigation and decision.

Disciplinary Actions

Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a written warning to ejection from the meeting or activity in question without refund of registration fees, being banned from participating in future Society meetings or Society-sponsored activities, being expelled from membership in the Society, and reporting the behavior to their employer or calling the authorities. In the event that the individual is dissatisfied with the results of the investigation, they may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

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GENERAL INFORMATION

Registration/Information Location and Hours

On Monday, Tuesday, Wednesday, and Thursday, registration will be in the Sibson Foyer, Level 1 of the Sibson Building. Registration hours are as follows:

Monday, July 17	8:15 - 20:00
Tuesday, July 18	8:15 - 14:45
Wednesday, July 19	8:15 - 18:00
Thursday, July 20	8:15 - 12:45

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available within the Sibson Building Lecture Hall SIB L2. Speakers are required to bring their own laptops and adaptors. MAC users are recommended to bring a laptop that supports HDMI connections. The university will provide HDMI cords, so presenters will not need to bring their own. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held in Sibson Foyer, Level 1.

2) A display board measuring 120 cm wide x 150 cm high - Portrait Style (approximately 3.9 feet wide x 4.9 feet high) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.

3) Posters should be set up on the morning of Monday, July 17 and removed by noon Thursday, July 20. All posters are available for viewing during all poster sessions; however, there will be formal poster presentations at the following times:

Monday, July 17	16:30 - 18:00
Tuesday, July 18	13:15 - 14:45
Wednesday, July 19	16:30 - 18:00

4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be disposed.

Meals and Coffee Breaks

- Breakfast is available at Dolche Vita, Keynes College for attendees staying in single/double accommodations in Keynes College.
- The Welcome Reception on Monday evening from 18:45 is in K-Bar, Keynes College.
- Coffee Breaks (Monday, Tuesday, Wednesday, and Thursday) will be served in Sibson Foyer, Level 1.
- Lunches (Monday, Tuesday, Wednesday, and Thursday) will be served in Sibson Foyer, Level 1
- Wednesday Banquet will be held at Socialite Rooftop & Bar beginning at 18:30

Smoking

Please be advised that the University of Kent operates a no smoking policy in all areas.

Name Badges

Name badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the meeting.

Internet Access

Delegates can access the University Guest Wi-fi using the i-cloud. All daytime session rooms and communal areas have free high speed Wi-Fi access. It is a condition of connecting to the internet using the Kent network that all laptops should be running current anti-virus software and the latest security updates.

Campus Map

Please click here for a map of the campus.

Cashless Campus

All receptions, cafes, and facilities on campus are cashless. We accept payment by debit/credit card only. If required, ATMs are available on the Jarman Plaza.

Places to Eat on Campus

Please see here for Vacation Catering giving details of catering outlets open during the meeting.

University Medical Centre

If you have a minor medical complaint, please contact the Medical Centre on campus: 01227469333. They are open Mon.-Fri. 8:00 – 18:30. If you require medical advice while the centre is closed, please ring the Out of Hours Service on 111. In the case of a medical emergency, please ring 999.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from July 17–20 during registration hours.

After registration hours please contact:

Dorothy Chaconas, BPS Staff
dchaconas@biophysics.org

Maija Ibanez, BPS Staff
mibanez@biophysics.org

Towards a More Perfect Union: Multi-Scale Models of Muscle and Their Experimental Validation

Canterbury, England

July 17—20, 2023

All scientific sessions will be held in the Sibson Building at the University of Kent Lecture Hall SIB L2, unless otherwise noted.

PROGRAM

Monday, July 17, 2023

7:45 – 20:00	Registration/Information	**Sibson Foyer**
8:15 – 8:30	Michael Regnier, University of Washington, USA Michael Geeves, University of Kent, United Kingdom ***Welcome and Opening Remarks***	
Session I	**MD/BS Simulations of Myofilament Proteins** Co-Chairs: Anne Houdusse, Institut Curie, France Matthew Childers, University of Washington, USA	
8:30 – 8:50	Anne Houdusse, Institut Curie, France ***Small Molecules Modulating Force Production: A New Perspective Against Muscle-Associated Diseases***	
8:50 – 9:10	Matthew Childers, University of Washington, USA ***Using 2'-Deoxy-ADP to Probe Stability of the Myosin Interacting Heads Motif at Atomic Resolution***	
9:10 – 9:30	Ian Gould, Imperial College, United Kingdom ***An In-Silico Investigation of the Effects of Small Molecules that Restore the Effects of Phosphorylation to Uncoupled Thin Filaments due to Cardiomyopathy-causing TNC G1590D Mutation***	
9:30 – 9:50	Michael Rynkiewicz, Boston University, USA ***Modeling the Troponin Core Domain on Thin Filaments Using Data from Cryoelectron Microscopy and Fluorescence Approaches***	
9:50 – 10:00	Brett Colson, University of Arizona, USA* ***Hypertrophic Cardiomyopathy Mutations in Cardiac Myosin-Binding Protein CN-Terminal Domains Cause Local and Allosteric Effects on Protein Mobility and Increase Actin Binding***	
10:00 – 10:30	**Coffee Break**	**Sibson Foyer**
Session II	**Integrating Computational Models at Different Spatial and Temporal Scales** Co-Chairs: Daniela Valdez-Jasso, University of California, San Diego, USA Joseph Powers, University of Washington, USA	

10:30 – 10:50	Daniela Valdez-Jasso, University of California, San Diego, USA ***Multi-scale Modeling for Determining Sex Differences in Resting and Active Myocardial Material Properties in a Rat PAH Model***
10:50 – 11:10	Joseph Powers, University of Washington, USA ***Integrating Multiscale Computational Models and Experimental Biomechanics to Investigate the Contractility Filamin C Deficient Hearts***
11:10 – 11:30	Srba Mijailovich, Filamentech, USA ***Integration of Multiple Experiments by Multiscale Computational Modeling***
11:30 – 11:50	Kyoko Yoshida, University of Minnesota, USA ***Multiscale Model Predictions of Heart Growth Applied to Postpartum and Hypertensive Pregnancies***
11:50 – 12:00	Amadeus Gebauer, Technical University of Munich, Germany* ***A Constrained Mixture Model of Sarcomere Turnover in Cardiomyocytes for Organ-Scale Cardiac Growth and Remodeling***
12:00 – 13:00	**Lunch** **Sibson Foyer**
Session III	**Do We Have an Accurate Measure of Ca^{2+} Transients – Does it Matter?** Co-Chairs: Ana Maria Gomez, INSERM, France Josine deWinter, Amsterdam, UMC, The Netherlands
13:00 – 13:20	Ana Marie Gomez, INSERM, France ***CA^{2+} Dynamics in Cardiac Pathological Models***
13:20 – 13:40	Josine deWinter, Amsterdam UMC, The Netherlands ***KBTBD13 is a Novel Cardiomyopathy Gene***
13:40 – 14:00	Corrado Poggesi, University of Florence, Italy ***Excitation-Contraction Coupling Alterations in Hypertrophic Cardiomyopathy and Arrhythmia Propensity***
14:00 – 14:20	Leonardo Sacconi, European Laboratory for Non-Linear Spectroscopy, Italy ***Correlating Electrical Disfunction and Structural Remodeling in Arrhythmogenic Mouse Hearts by Advanced Optical Methods.***
14:20 – 14:30	Sage Malingen, University of Washington, USA* ***Molecular Dynamics Simulations Reveal Functional Changes in Troponin Resulting from Mutations Implicated in Cardiomyopathies***
14:30 – 15:00	**Coffee Break** **Sibson Foyer**
Session IV	**W*hat's New in Thin Filament Regulation and What Cryo-EM Doesn't Tell Us*** Co-chairs: Jil Tardiff, The University of Arizona, USA Ivanka Sevrieva, King's College of London, United Kingdom
15:00 – 15:20	Jil Tardiff, The University of Arizona, USA ***Walking a Thin (Filament) Line: Integrating Time-Resolved Fret and Computation to Identify Novel Pathogenic Mechanisms in Hypertrophic Cardiomyopathy***

15:20 – 15:40	Ivanka Sevrieva, Kings College London, United Kingdom ***Comparison of the Cardiac Troponin Conformation Determined by Polarized Fluorescence and Cryo-EM***	
15:40 –16:00	William Lehman, Boston University, USA TROPONIN-I Induced Tropomyosin Pivoting Defines Cardiac Thin Filament Structure in Relaxed Muscle	
16:00 – 16:20	Yuichiro Maeda, Okayama University, Japan ***Structures and Mechanisms of Actin ATP Hydrolysis***	
16:20 – 16:30	Joseph Chalovich, ECU Brody School of Medicine, USA* ***The Unstructured C-Terminal Region of Troponin T Retards Calcium Activation of Striated Muscle***	
16:30 – 18:00	**Poster Session**	**Sibson Foyer**
18:00 – 18:45	**Keynote Address** Steven Schwartz, The University of Arizona, USA **Model Building Challenges and the Time-Scale Problem in the Computational Study of Muscle Biophysics**	
18:45	**Reception**	**Keynes/K-Bar**

Tuesday, July 18, 2023

8:15 – 14:45	**Registration/Information**	**Sibson Foyer**
8:15 – 9:00	**Keynote Address** Leslie Leinwand, University of Colorado, USA ***Multiscale Regulation of Sarcomeres in Health and Disease***	
Session V	**What do Modelers Need from Experimentalists and Vice Versa?** Chair: Malcolm Irving, Kings College London, United Kingdom	
9:00 – 9:15	Stuart Campbell, Yale University, USA ***Predicting Clinical Phenotypes of TPM1 Missense Mutations***	
9:15 – 9:30	Martin Pfaller, Stanford University, USA ***FSGE: A Computational Model for Equilibrated Cardiovascular Fluid-Solid-Growth Interaction***	
9:30 – 10:30	**Panel Discussion** Chair: Malcolm Irving, King's College London, United Kingdom	

10:30 – 10:45	**Coffee Break**	**Sibson Foyer**
Session VI	**Small Molecule Therapeutics for Muscle Disease** Chair: Michael Regnier, University of Washington, USA	
10:45 –11:05	Michael Regnier, University of Washington, USA ***Experimental and Computational Approaches for Mechanistic Analysis and Therapeutic Development of 2-Deoxy ATP to Treat Heart Failure***	
11:05 – 11:25	Allan Russell, Edgewise Therapeutics, USA ***Modulating Fast Skeletal Muscle Contraction as a Novel Strategy for the Protection of Skeletal Muscle in Muscular Dystrophy***	
11:25 – 11:40	James Hartman, Cytokinetics, USA ***Preclinical Characterization of CK-4021586, A New Class of Cardiac Myosin Inhibitors for the Treatment of Hypertrophic Cardiomyopathy***	
11:40 – 12:00	Andras Malnasi-Csizmadia, Evötös Loránd University, Hungary ***Drug Design Targeting Myosin Blebbistatin Binding Site***	
12:00 – 12:10	Marcus Hock, University of California, San Diego, USA* ***Multiscale Simulations of the Effects of 2'-Deoxy-ATP and Myosin Mutations on Actomyosin Interactions***	
12:15 – 13:15	**Lunch**	**Sibson Foyer**
13:15 – 14:45	**Poster Session II**	**Sibson Foyer**

Wednesday, July 19, 2023

8:00 – 18:00	**Registration/Information**	**Sibson Foyer**
8:15 – 8:45	**Keynote Address** Elisabetta Brunello, King's College of London, United Kingdom ***Structural Dynamics of the Thick Filament During the Physiological Cardiac Cycle***	
Session VII	**What's New in Thick Filament Regulation** Co-Chairs: Samantha Harris, The University of Arizona, USA Christopher Toepfer, University of Oxford, United Kingdom	
8:45 – 9:05	Samantha Harris, The University of Arizona, USA ***Cut and Paste of Myosin Binding Protein-C in Skeletal Muscles***	
9:05 – 9:25	Christopher Toepfer, University of Oxford, United Kingdom ***Understanding Hypertrophic Cardiomyopathy (Hcm) Across the Thick and Thin Filament, Bridging In-Vitro and In-Silico Models of Disease to Accelerate Pathomechanism Discovery***	
9:25 – 9:45	Thomas Irving, Illinois Institute of Technology, USA ***Myosin Based Regulation: The Plot Thickens***	

9:45 – 10:05	Neil Kad, University of Kent, United Kingdom ***Single Molecule Imaging Approaches Towards Understanding Dual Filament Regulation in Muscle***
10:05 – 10:15	Charles Chung, Wayne State University, USA* ***Ramp-Stretches During Relaxation of Twitching Intact Cardiac Traecula Suggest a Need for Dynamic Models of Myofilament Function***
10:15 – 10:45	**Coffee Break** **Sibson Foyer**
Session VIII	**Cross-Bridge Recruitment and Dynamics: New and Unresolved Questions** Co-chairs: Michael Geeves, University of Kent, United Kingdom Manta Amrute Nayak, Medical School of Hanover, Germany
10:45 – 11:05	Michael Geeves, University of Kent, United Kingdom **Are the DRX and SRX States in Thermal Equilibrium a Paradox?**
11:05 – 11:25	Mamta Amrute-Nayak, Medical School of Hanover, Germany ***Evidence for Two Distinct Actomyosin Cross-Bridge Stiffness for Human Beta Cardiac Myosin***
11:25 – 11:45	David Warshaw, University of Vermont, USA ***Myosin-Binding Protein C/H: Impact on Muscle Contractility and Development***
11:45 – 12:05	Edward DeBold, University of Massachusetts, USA ***Recent Biophysical Approaches to Investigate the Coupling Between Force-Generation and Phosphate-Release in Myosin***
12:05 – 12:15	Matvey Pilagov, University of Kent, United Kingdom* ***Super-Resolution Single-Molecule Imaging of ATP Usage in Myofibrils to Study Thick Filament Regulation***
12:15 – 13:15	**Lunch** **Sibson Foyer**
Session IX	**What is on the Horizon from Modellers and Experimentalists?** Chair: Andrew McCulloch, University of California San Diego, USA
13:15 – 13:30	Andrew McCulloch, University of California, San Diego, USA ***Multi-Scale Modeling of Biophysics: From Molecules to Populations***
13:30 – 13:45	Farah Sheikh, University of California, San Diego, USA ***Connexin43 Restoration Alleviates End-Stage Alterations in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy***
13:45 – 14:30	Panel Discussion Chair Andrew McCulloch, University of California, San Diego, US
14:30 – 15:00	**Coffee Break** **Sibson Foyer**
Session X	**Passive Tension in Muscle Cells: Protein Players and Plasticity** Co-chairs: Jolanda Van der Veldon, Amsterdam University Matthew Caporizzo, University of Vermont, USA
15:00 – 15:20	Jolanda Van der Veldon, Amsterdam University, The Netherlands ***Modulators of Cardiac Muscle Relaxation and their Role in Cardiac Pathology***

15:20 – 15:40	Matthew Caporizzo, University of Vermont, USA ***Microtubules are Viscous Regulators of Myocardial Motion***	
15:40 – 16:00	Michael Gotthardt, Max Delbruck Center, Germany ***Regulating Titin Based Stiffness in Health and Disease***	
16:00 – 16:20	Jennifer Davis, University of Washington, USA ***Fibroblast-Mediated Regulation of Cardiac Structure and Stiffness***	
16:20 – 16:30	Bradley Palmer, University of Vermont, USA* ***Contribution of Thick Filament Stiffness to Length Dependent Activation in Cardiac Muscle***	
16:30 – 18:00	**Poster Session III**	**Sibson Foyer**
18 :30	**Dinner Banquet**	**Socialite Restaurant**

Thursday, July 20, 2023

8:00 – 12:45	**Information**	**Sibson Foyer**
8:15 – 8:45	**Keynote Address** Thomas Daniel, University of Washington, USA ***Multiscale Models of Muscle Melding Molecular and Myofilament Levels of Organization***	
Session XI	**Sarcomere and Muscle Cell Models of Contractile Dynamics** Co-chairs: Kenneth Campbell, University of Kentucky, USA Cheavar Blair, University of California, Santa Barbara, USA	
8:45 – 9:05	Kenneth Campbell, University of Kentucky, USA ***Multiscale Modeling of Pathological Cardiac Growth***	
9:05 – 9:25	Cheavar Blair, University of California, Santa Barbara, USA **Sarcomere Dynamics Simulation to Uncover Mechanisms in Hypertrophic Cardiomyopathy**	
9:25 – 9:45	Michael Greenberg, Washington University in St. Louis, USA ***Harnessing Multiscale Models to Understand Dilated Cardiomyopathy Thin Filament Mutations***	
9:45 – 10:05	Farid Moussavi-Harami, University of Washington, USA ***Machine Learning for Building Classifiers and Rate Estimates in Simulated Twitches***	
10:05 – 10:15	Howard White, Eastern Virginia Medical Center Physiological Sciences, USA* ***Structure of Prepowerstroke Actomyosin by Cryoelectronmicroscopy at 10MD and 5A***	
10:15 – 10:45	**Coffee Break**	**Sibson Foyer**

Session XII	**Upscale Modeling: Myofilaments to Movement** Co-Chairs: Silvia Blemker, University of Virginia, USA Kissa Nishikawa, Northern Arizona University, USA
10:45 – 11:05	Silvia Blemker, University of Virginia, USA ***Multi-Scale Modeling Across Muscles, Contexts, and Diseases***
11:05 – 11:25	Kiisa Nishikawa, Norther Arizona University, USA ***Multiscale Muscle Modeling: An Organismal Approach***
11:25 – 11:45	Madhusudhan Venkadesan, Yale University, USA ***Emergent Rheology of Actomyosin Ensembles***
11:45 – 12:05	Anne Silverman, Colorado School of Mines, USA ***Modeling Muscle Coordination in Whole Body Movement***
12:05 – 12:15	Bertrand Tanner, Washington State University, USA* ***An Experimental Approach to Manipulate the Multi-Scale Components of Whole-Body Movement that Influence Single Muscle Fiber Work and Power Output***
12:15 – 12:45	**Closing Remarks and *Biophysical Journal* Poster Awards**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

SMALL MOLECULES MODULATING FORCE PRODUCTION: A NEW PERSPECTIVE AGAINST MUSCLE-ASSOCIATED DISEASES

Anne Houdusse[1]
[1]Institut Curie, Paris, France

Myosins are ATP-dependent molecular motors involved in almost all processes of life. These motors are associated with various human diseases such as various cardiomyopathies, spasticity, deafness or malaria. Nowadays, the most promising approach to treat myosin-associated diseases is the design of small-molecule drugs able to specifically modulate the force produced by these motors. Camzyos (mavacamten) is a specific inhibitor recently approved by the FDA to treat adults with symptomatic class II-III obstructive hypertrophic cardiomyopathy. Omecamtiv mecarbil (OM) also targets β-cardiac myosin but in this case, it is an activator of contraction, currently in late Phase 3 for heart failure. Here we present how combining X-ray crystallography, molecular dynamics and functional assays allows to study the mechanism of action of some of these compounds. We have determined that despite their antagonistic effects on force production, OM and Mava surprisingly target the same pocket. MPH-220 is a Blebbistatin derivative identified as an inhibitor of skeletal myosin-2 (SkMyo2) and a promising treatment against muscle spasticity. Structures of other inhibitors bound in this pocket with distinct IC50 for three muscle myosins have also provided clues about the parameters that control potency and specificity. Altogether, these results show how small molecules modulating the force produced by myosins are promising avenues to treat myosin-associated diseases and opens up new approaches to the design of more selective and potent compounds.

USING 2'-DEOXY-ADP TO PROBE STABILITY OF THE MYOSIN INTERACTING HEADS MOTIF AT ATOMIC RESOLUTION

Matthew C. Childers; Michael Regnier[1];
[1]University of Washington, Bioengineering, Seattle, WA, USA, WA, USA

In addition to active cycling states, myosins within the thick filament can access an 'inactive' conformation called the interacting heads motif (IHM) that has been associated with the energy-conserving super relaxed (SRX) state of muscle. Thus, accessing the IHM conformation is a means of thick filament-based regulation of muscle. Only a handful of cryo-EM structures of IHM myosin are available. The large size of the IHM has similarly challenged all-atom molecular simulations of the structure. have performed explicit solvent, all-atom molecular dynamics simulations of human cardiac b-myosin in the IHM confirmation on the microsecond timescale. In recent experimental studies, the small molecule ATP analogue, 2'-deoxy-ATP (dATP), has been shown to destabilize myosin heads from the IHM into disordered, more active states. To complement these experimental studies, we simulated b-myosin in the IHM conformation in which ADP.P_i was replaced by dADP.P_i. These simulations showed that dADP reduced the stability of the IHM by reducing the number of interactions between S1 heads as well as the net interaction energy between the heads. They also show that the tails of dADP.P_i -bound heads adopted conformations distinct from existing atomic models obtained with cryoEM. Thus, simulations suggest that dynamics in the RLC-binding region of the tail and at the head-head interface both contribute to IHM stability. Further, they suggest that departure from the IHM state involves coordinated motions in regions of myosin separated by over 100 Å. These novel simulations should also prompt further research into the contribution of tail dynamics into IHM stability as well as interest in mutations that influence tail dynamics. Ongoing coarse-grained simulations will probe the stability of the interacting heads over longer timescales.

AN IN-SILICO INVESTIGATION OF THE EFFECTS OF SMALL MOLECULES THAT RESTORE THE EFFECTS OF PHOSPHORYLATION TO UNCOUPLED THIN FILAMENTS DUE TO CARDIOMYOPATHY-CAUSING TNC G159D MUTATION.

Ian R Gould[1]; Steve B Marston[2]; Tony Yang[1];
[1]imperial college london, Chemistry, London, United Kingdom
[2]Imperial College London, National Heart and Lung Institute, London, United Kingdom

Cardiac muscle possesses a unique modulatory mechanism that allows the heart to meet increased oxygen demand during exercise. Adrenergic activation of PKA targets the sarcolemma, sarcoplasmic reticulum and contractile apparatus to increase contractile force and heart rate. In the thin filaments of the contractile apparatus, cTnI Ser22 and Ser23 in the cardiac-specific N-terminal peptide (NcTnI: residues 1 to 32) are the targets for PKA phosphorylation. The effect of phosphorylation is a 2-3 fold decrease of affinity of cTn for Ca^{2+}due to altered cTnC-cTnI interactions, linked to a higher rate of Ca^{2+} dissociation from cTnC leading to a faster relaxation rate of the cardiac muscle (lusitropy). Cardiomyopathy-linked mutations primarily affect Ca^{2+} regulation or the PKA-dependent modulatory system, such that Ca^{2+}-sensitivity becomes independent of phosphorylation level (uncoupling) and this could be sufficient to induce cardiomyopathy.A drug that could restore the phosphorylation-dependent modulation of Ca^{2+}-sensitivity could have potential for treatment of these pathologies. We have found that in single filament assays that a number of small molecules including SilybinB, Resveratrol and EGCG can restore coupling. We performed Molecular Dynamics (MD) simulations of the unphosphorylated and phosphorylated cardiac Troponin core with the G159D DCM mutation. We found that SilybinB, EGCG and resveratrol restored most metrics to wild-type values, whilst SilybinA, an inactive isomer of SilybinB, did not. We analysed the atomic-level changes induced by ligand binding to explain recoupling. The changes induced by small molecules are all consistent between various measurement techniques from the atomic to the cellular level.

MODELING THE TROPONIN CORE DOMAIN ON THIN FILAMENTS USING DATA FROM CRYOELECTRON MICROSCOPY AND FLUORESCENCE APPROACHES

Michael J Rynkiewicz[1]; Ivanka Sevrieva[2]; Malcolm Irving[2]; William Lehman[1];
[1]Boston University Chobanian & Avedisian School of Medicine, Pharmacology, Physiology, & Biophysics, Boston, MA, USA
[2]King's College London, Randall Centre for Cell & Molecular Biophysics, London, United Kingdom

Our understanding of thin filament structure has been greatly enhanced by the publication of a number of models derived from cryo-electron microscopy studies (Yamada et al. (2020) and then later confirmed by Risi et al. (2021)). These studies elucidated atomic level details of the calcium-dependent activation of the thin filament leading to force development in cardiac muscle. However, while cryo-EM reconstruction is best suited to capture the high-resolution organization of static structures, troponin and tropomyosin are dynamic components of the thin filament and corresponding disorder is not always easily recorded and classified by the method. For example, the N-lobe of cardiac muscle troponin subunit C (TnC), which binds calcium and the troponin subunit I switch peptide during thin filament activation, has been shown to take up multiple distinct orientations in studies of polarized fluorescence of TnC labeled with bifunctional rhodamine (Sevrieva et al., 2014). Here, we generate small ensembles of structures to incorporate known sources of disorder as guided by information derived from multiple biophysical techniques. We use orientational data derived both from both polarized fluorescence and cryo-EM methodologies to guide molecular dynamics simulations to build a set of unique models that satisfy both sets of data. Analysis of these structures shows the details of how the dynamic nature of the N-lobe of TnC is important for its function in thin filament regulation.

HYPERTROPHIC CARDIOMYOPATHY MUTATIONS IN CARDIAC MYOSIN-BINDING PROTEIN C N-TERMINAL DOMAINS CAUSE LOCAL AND ALLOSTERIC EFFECTS ON PROTEIN MOBILITY AND INCREASE ACTIN BINDING

Rhye-Samuel Kanassatega[1]; Fiona L Wong[1]; Thomas A Bunch[1]; L. Michel Espinoza-Fonseca[2]; **Brett A Colson**[1];
[1]University of Arizona, Cellular & Molecular Medicine, Tucson, AZ, USA
[2]University of Michigan, Internal Medicine, Ann Arbor, MI, USA

Mutations in the gene encoding cardiac myosin-binding protein C (cMyBP-C) are a leading cause of hypertrophic cardiomyopathy (HCM). HCM affects more than 1 in 500 individuals and is a leading cause of death in young individuals. However, the mechanisms leading to cardiac dysfunction in HCM remain unclear and therapies are limited. We have used molecular dynamics (MD) simulations and biochemical and biophysical approaches to gain insight into the molecular mechanisms by which mutations in cMyBP-C cause HCM disease. cMyBP-C N-terminal domains C0 through C2 (C0-C2) are considered to be the "business end" of the molecule as they contain regions for binding actin and myosin and phosphorylation, which are important for cMyBP-C's critical roles in normal cardiac function. We selected three HCM-causing mutations in C0-C2 expected to be pathogenic according to the SHaRe Registry and NIH ClinVar databases to investigate: P161S and Y237S in the hydrophobic core of domain C1 and surface-exposed P371R in domain C2. Using a fluorescence lifetime-based actin-binding assay, we determined that all 3 HCM mutations increased in vitro binding affinity for actin in both unphosphorylated and phosphorylated C0-C2. We also found that the root mean square fluctuation (RMSF) values used to measure the in silico mobility of the protein in the trajectories (a total of 4 ms each) at either 25 or >50 ºC induced moderate local structural changes in P161S and P371R and allosteric structural changes in Y237S as compared to immunoglobulin-like wild type C1 or C2 domain structures. In addition, mutations reduced in vitro protein folding stability of C0-C2, C1, and C2 relative to wild type, as expected, using differential scanning calorimetry (DSC) and protein solubility assays. These in vitro and in silico results suggest that structural changes in mutant cMyBP-C domains can cause altered myofilament binding, and thereby lead to dysfunction and HCM disease.

MULTISCALE MODELING FOR DETERMINING SEX DIFFERENCES IN RESTING AND ACTIVE MYOCARDIAL MATERIAL PROPERTIES IN A RAT PAH MODEL

Daniela Valdez-Jasso[1];
[1]University of California San Diego, Bioengineering, La Jolla, CA, USA

Pulmonary arterial hypertension (PAH) is caused by adverse idiopathic remodeling of the pulmonary arteries, is ~3X more prevalent in women, and results in 5-year survival <50% due to right-ventricular (RV) failure. There are no therapies to prevent RV failure or reverse vascular remodeling in PAH. We use multi-scale experimental and computational modeling that spans from organ-scale in vivo physiology and hemodynamics to tissue-scale structural and mechanical analysis and molecular studies of cell mechano-signaling. In sugen-hypoxia rats, our in-vivo measurements and computational models show that while the male RV relies on hypertrophy to maintain compensated systolic function, female rats recruit increased myocyte contractility and hypertrophy less. While male rats developed increased filling pressures and profound myocardial matrix stiffening, female rats were protected from this fibrotic remodeling. In isolated cardiac myocytes, calcium handling had a higher functional reserve in females compared with males, and RV fibroblasts show distinct mechano-signaling responses from left-ventricular cells. These experimental and computational results suggest a new paradigm in the pathophysiology of PAH and key differences in mechanisms and outcomes between males and females.

INTEGRATING MULTISCALE COMPUTATIONAL MODELS AND EXPERIMENTAL BIOMECHANICS TO INVESTIGATE THE CONTRACTILITY FILAMIN C DEFICIENT HEARTS

Joseph D Powers[1,2];
[1]University of Washington, Laboratory Medicine and Pathology, Seattle, WA, USA
[2]University of Washington, Mechanical Engineering, Seattle, WA, USA

Dilated cardiomyopathy (DCM) is a common and deadly form of heart disease that is typically characterized by progressive thinning of ventricular walls, chamber dilation, and systolic dysfunction. DCM is often associated with mutations in genes encoding sarcomere or cytoskeleton proteins that confer contractile dysfunction and adverse cellular remodeling via poorly understood mechanisms. One such protein is Filamin C (FLNC), which interacts with multiple proteins in the Z-disc and the costamere, suggesting that it is important for maintaining those structures and contributing to mechanical force transmission in the heart. Moreover, many mutations in the gene that encodes FLNC are associated with multiple forms of human cardiomyopathies, with many unique FLNC mutations found in patients with DCM. However, the mechanisms that lead to DCM in patients with FLNC variants are not known. The objective of this study was to elucidate mechanisms by which FLNC regulate systolic force transmission in the heart and how a loss of functional FLNC drives progressive DCM. To do so, we used a genetically engineered mouse model that enables inducible homozygous knockout of FLNC (FLNC-KO) in adult mice, which triggers a rapid DCM phenotype. Experimental biomechanics using single cardiomyocytes and papillary muscles isolated from FLNC-KO and control adult mouse hearts revealed a loss of contractility, but no effects on calcium signaling in FLNC-KO hearts compared to controls. Quantitative electron microscopy and immunofluorescent microscopy techniques were used to inform spatially explicit computational models of sarcomere/cell mechanics, which, together, revealed that a loss of FLNC induces adverse structural adaptations at the myofibril level that contribute to disrupted longitudinal force production during contraction. This work provides new insights into the pathological mechanisms by which dysfunctional FLNC promotes systolic abnormalities, subcellular remodeling, and the development of DCM.

INTEGRATION OF MULTIPLE EXPERIMENTS BY MULTISCALE COMPUTATIONAL MODELING

Srboljub M Mijailovich[1]; Momcilo Prodanovic[1,2,3]; Michael Regnier[4]; Corrado Poggesi[5]; Thomas C Irving[6]; Michael A Geeves[7]; [1]FilamenTech, Inc, Newton, MA, USA
[2]University of Kragujevac, Faculty of Engineering, Kragujevac, Serbia
[3]Institute for Information Technologies. University of Kragujevac, Technical and Technological Sciences, Kragujevac, Serbia
[4]University of Washington, Bioengineering, Seattle , WA, USA
[5]University of Florence, Experimental & Clinical Medicine, Florence, Italy
[6]Illinois Institute of Technology, Biology, Chicago, IL, USA
[7]University of Kent, Biosciences, Canterbury, United Kingdom

Research on the structure and function of muscle and how this relates to muscle disease has generated a vast quantity of data spanning multiple spatial and temporal scales differing by up to ten orders of magnitude. A major difficulty, however, is relating these valuable bits of information from different experimental setups to relevant in vivo observations for assessing disease progression or the effectiveness of appropriate drugs and treatments. Recent multiscale computational modeling approaches that integrate information from a wide range of experiments can provide a deeper understanding of muscle functionality and its impact on human health. Spatially explicit stochastic models such as MUSICO platform enable translation of data from sub-molecular structural and biochemical information to muscle fiber dynamic functional behavior. The platform provides a powerful tool for quantitatively assessing the consequences of mutations in sarcomeric proteins, and the effects of Ca^{2+} and small molecule therapeutics by incorporating detailed 3D structural information, crossbridge cycling kinetics, thin and thick filament regulation, and effect of accessory proteins including titin, nebulin and MyBP-C. This modeling approach successfully quantified the effects of (1) mutations in regulatory protein troponin-C and myosin; (2) differences in myosin isoforms across species, including humans; (3) transitions to and from an inactive myosin "parked state" governed by [Ca^{2+}] and thick filament sensitivity to force; role of (4) nebulin in nemaline myopathy; (5) roles of titin and interfilament spacing in length dependent activation; (6) interactions of MyBP-C with actin filaments and myosin in hypertrophic cardiomyopathy; and (7) the effects of drugs including mavacamten, 2-deoxyATP, disopyramide and digoxin. The mechanisms of these molecular modulations when translated to muscle fibers can be used for simulations of whole organ function using Finite Element solvers where instantaneous muscle material characteristics are assessed via computationally effective surrogate models.

MULTISCALE MODEL PREDICTIONS OF HEART GROWTH APPLIED TO POSTPARTUM AND HYPERTENSIVE PREGNANCIES

Molly Kaissar[1]; **Kyoko Yoshida**;
[1]University of Minnesota - Twin Cities, Biomedical Engineering, Minneapolis, MN, USA

Pregnancy stands at the interface of mechanics and biology. The growing fetus combined with a surge in circulating hormones induce rapid growth and remodeling of the maternal cardiovascular system. We previously developed a multiscale computational model that incorporates hormonal and hemodynamic changes during pregnancy to successfully predict left ventricular (LV) growth in rats. Here, we assess our model's ability to predict heart growth after delivery and in hypertensive pregnancies. Our multiscale model couples a cell-signaling model that predicts cardiomyocyte hypertrophy in response to reproductive and cardiovascular hormones, as well as a mechanotransduction pathway, to a mechanics-based model of the rat heart and circulation that predicts organ-level LV growth in response to hemodynamic changes. We simulated 21 days of pregnancy, followed by 21 days of postpartum changes in hemodynamics and hormones. We investigated both non-lactating and lactating rats, since they exhibit differences in postpartum hormones and hemodynamics. In addition, we simulated three cases of pregnancy with superimposed hypertension: Angiotensin II infusion (+AngII), transverse aortic constriction (+TAC), and reduced uterine perfusion pressure (+RUPP). Our model predicted LV mass decrease in non-lactating rats, while the elevated hormones and cardiac output led to LV mass increase in lactating rats, consistent with the available literature. Our hypertension simulations correctly captured LV growth in +AngII and +TAC during pregnancy but could not capture reported growth in the +RUPP group. Discussion: Our multiscale model correctly captured cardiac growth during postpartum and in 2 of 3 cases of hypertensive pregnancies. Our analysis indicates a key hormonal role in cardiac growth during hypertensive pregnancies. In contrast, our model indicated dynamic mechanical signals on cardiomyocytes during postpartum, suggesting a key mechanical role in driving heart growth after delivery. We are currently improving our model to include other key hormones to improve our predictions, specifically for +RUPP pregnancies.

A CONSTRAINED MIXTURE MODEL OF SARCOMERE TURNOVER IN CARDIOMYOCYTES FOR ORGAN-SCALE CARDIAC GROWTH AND REMODELING

Amadeus M Gebauer[1]; Martin R Pfaller[2,3,4]; Wolfgang A Wall[1];
[1]Technical University of Munich, Institute of Computational Mechanics, Munich, Germany
[2]Stanford University, Pediatric Cardiology, Stanford, CA, USA
[3]Stanford University, Stanford Maternal & Child Health Research Institute, Stanford, CA, USA
[4]Stanford University, Institute for Computational and Mathematical Engineering, Stanford, CA, USA

Changes in the mechanical environment in the heart wall (e.g., caused by cardiovascular diseases) trigger cardiac growth and remodeling (G&R), eventually leading to heart failure in many patients. An important mechanism of G&R in soft tissue is turnover, which is the continual deposition and degradation of tissue constituents. We propose a novel model of sarcomere turnover in cardiomyocytes based on the constrained mixture model and a rheological model of sarcomeres. The rheological model of the sarcomeres includes passive and active contributions. Sarcomeres continuously replace themselves or even grow or shrink in number when cardiomyocytes are perturbed from their preferred mechanical environment. Existing models often do not explicitly model the effect of turnover on the active part of the tissue. We combine our novel sarcomere turnover model with the existing G&R model of the extracellular matrix in our finite-element-based simulation framework. We apply our model to a patient-specific bi-ventricular heart and show how changes in the mechanical environment of cardiomyocytes induced by different overload conditions result in organ-scale G&R. We identify mechanobiological stable and unstable growth depending on the severity of hypertension and different growth factors. Furthermore, we elaborate on how our model predicts the reversal of G&R after returning blood pressure to baseline. Our microstructure-motivated model of organ-scale cardiac G&R, together with experimental data from biomimetic cultures of living human myocardium and clinical data of long-term cardiac magnetic resonance imaging of patients has the potential to not only increase understanding but also identify patients at risk of heart failure and assess or even improve their personalized therapy options.

CA^{2+} DYNAMICS IN CARDIAC PATHOLOGICAL MODELS

Ana Maria Gomez
INSERM, France

No Abstract

KBTBD13^{R408C}-KNOCKIN MOUSE MODEL REVEALS IMPAIRED RELAXATION KINETICS AS NOVEL PATHOMECHANISM FOR NEM6 CARDIOMYOPATHY

Rianne J Baelde[1]; Valentijn J Janssen[1]; Alexcia Fortes-Monteiro[1]; Ricardo A Galli[1]; Sylvia Bogaards[1]; Mei Methawasin[2]; Diederik Kuster[1]; Jolanda van der Velden[1]; Coen Ottenheijm[1,2]; **Josine M de Winter**[1];
[1] Amsterdam UMC, location VUmc, Physiology, Amsterdam, The Netherlands
[2]University of Arizona, Dept. of Cellular and Molecular Medicine, Tucson, AZ, USA

A prime example of the impact of impaired relaxation kinetics is nemaline myopathy caused by variants in KBTBD13 (NEM6) encoding kelch repeat and BTB (POZ) domain containing 13. In addition to weakness, NEM6 patients have slowed muscle relaxation, compromising contractility and daily-life activities. The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (KBTBD13^{R408C}). Recently, we discovered that the Dutch founder variant not only affects skeletal muscle function, but also the heart. Patients display cardiac abnormalities including systolic dysfunction, diastolic dysfunction, atrial fibrillation, ventricular tachycardia and repolarization disturbances. Our studies on skeletal muscle provided insight in the sarcomere-based pathomechanism in NEM6: KBTBD13^{R408C} causes structural changes in the actin-based thin filament, increasing thin filament stiffness and impairing relaxation kinetics. To provide insight in the mechanism underlying cardiac dysfunction in NEM6, here we assessed cardiac structure and function in Kbtbd13^{R408C}-knockin mice, which closely recapitulate the skeletal muscle phenotype. Pressure-volume loop analyses showed that the end-systolic pressure-volume relation was unaffected, but the end-diastolic pressure-volume relation was steeper in Kbtbd13^{R408C}-knockin mice, indicating diastolic dysfunction. Histological evaluation of cardiac structure revealed no changes in fibrosis in Kbtbd13R408C-knockin mice. Also, no changes in titin isoform composition were found that could account for the increased diastolic stiffness. Next, we studied the contraction and relaxation kinetics at the intact cardiomyocyte level by a high-throughput contractility set-up: Percentage of shortening was not affected, however Time to baseline was increased in Kbtbd13^{R408C}-knockin mice, indicating impaired relaxation kinetics. In parallel, calcium-handling was assessed by calcium indicator Fura-2AM. These studies reveal that calcium-release is not affected, but calcium-reuptake is impaired in Kbtbd13^{R408C}-knockin mice, which might contribute to the impaired relaxation kinetics. Current studies focus on how KBTBD13R408C affects calcium-reuptake kinetics and sarcomere kinetics in NEM6 cardiomyocytes. Hence, our studies provide the first insights in the pathomechanism underlying cardiac dysfunction in NEM6.

EXCITATION-CONTRACTION COUPLING ALTERATIONS IN HYPERTROPHIC CARDIOMYOPATHY AND ARRHYTHMIA PROPENSITY

Raffaele Coppini[1]; Cecilia Ferrantini[1]; Lorenzo Santini[1]; Chiara Palandri[1]; Josè M Pioner[1]; Marianna Langione[1]; Chiara Tesi[1]; Elisabetta Cerbai[1]; Iacopo Olivotto[1]; **Corrado Poggesi**[1]; [1]University of Florence, Firenze, Italy

Hypertrophic Cardiomyopathy (HCM) is the most frequent inherited cardiomyopathy, often associated with sarcomeric protein mutations. In our long lasting collaboration with the Florence Cardiomyopathy Referral Center we performed biophysical investigations on a large number of preparations from surgical samples of HCM patients undergoing septal myectomy and compared the results with those of control hearts (either donors or samples from non-failing non hypertrophic hearts). We found that HCM mutations may primarily affect sarcomere function through different mechanisms but all mutations affect cardiomyocyte electrophysiology and Ca^{2+} transient due to remodeling. HCM cardiomyocytes always showed prolonged action potentials (APs) and slower Ca^{2+} transients. These changes were associated with a higher likelihood of early and delayed after-depolarizations (EADs and DADs). Among HCM samples, those which scored positive for EADs had longer AP duration as compared with those showing no EADs; moreover, samples from DAD-positive patients showed longer Ca^{2+}-transient decay with respect to DAD-negative samples. These observations confirm that EADs are associated with AP prolongation and DADs are linked with altered Ca^{2+} cycling. Clinical follow-up of the patients who had provided the cardiomyocytes used for experiments allowed us to establish a correlation between the presence of EADs and DADs in cardiomyocytes and the occurrence of clinical arrhythmias in the same patients. Patients who scored positive for EADs or DADs were more likely to experience arrhythmic episodes during follow up. Additional investigations in mouse and iPSC models of HCM allowed us to establish that (i) the severity of the HCM E-C coupling remodeling and its sequelae vary with the mutation and the related myofilament dysfunction; (ii) the HCM E-C coupling remodeling may be a compensation (though pro-arrhythmogenic) for the impact of the mutation on myofilament function; (iii) the HCM E-C coupling remodeling occurs early in the disease.

CORRELATING ELECTRICAL DISFUNCTION AND STRUCTURAL REMODELING IN ARRHYTHMOGENIC MOUSE HEARTS BY ADVANCED OPTICAL METHODS

Leonardo Sacconi;
[1]Institute of Clinical Physiology (IFC) - CNR, Florence, Italy
[2]Institute for Experimental Cardiovascular Medicine, Freiburg, Germany

Severe remodeling processes may occur in the heart due to both genetic and non- genetic diseases. Structural remodeling, such as collagen deposition (fibrosis) and cellular misalignment, can affect electrical conduction at different orders of magnitude and, eventually, lead to arrhythmias. In this scenario, arrhythmogenic cardiomyopathy (ACM) is an inherited heart disease that involves ventricular dysfunction, arrhythmias and localized replacement of contractile fibers with fibrofatty scar tissue. Unfortunately, nowadays, predicting the impact of fine structural alterations on the electrical dysfunction in entire organs is challenging, due to the inefficacy of standard imaging methods in performing high-resolution three-dimensional reconstructions in massive tissues. In this work, we developed a new fully optical correlative approach to quantify and integrate the electrical dysfunctions with three-dimensional structural reconstructions of entire hearts, both in controls and in a mouse model of ACM. We combined optical mapping of the action potential propagation (APP) with advances in tissue clearing and light-sheet microscopy techniques. First, we employed an optical platform to map and analyze the APP in Langendorff-perfused hearts. Then, we optimized the SHIELD procedure for the clearing of cardiac tissue, thus converting the previously electrically characterized samples into well-preserved and fully-transparent specimens. A high-throughput light-sheet microscope has been developed following the mesoSPIM project: the conceived microscope allows the reconstruction of the whole mouse heart with micrometric resolution allowing fine quantification of myocytes alignment and fibrosis deposition across the organ. Finally, we developed a software pipeline that employs high-resolution 3D images to analyze and co-register APP maps with the 3D anatomy, contractile fibers disarray, and fibrosis deposition on each heart. We believe that this promising methodological framework will allow clarifying the involvement of fine structural alterations in the electrical dysfunctions, thus enabling a unified investigation of the structural causes that lead to electrical and mechanical alterations after the tissue remodeling.

MOLECULAR DYNAMICS SIMULATIONS REVEAL FUNCTIONAL CHANGES IN TROPONIN RESULTING FROM MUTATIONS IMPLICATED IN CARDIOMYOPATHIES

Sage A Malingen[1,2]; Matthew C Childers[1,2]; Travis Tune[2,3]; Kerry Kao[1,2]; Thomas Daniel[2,3]; Farid Moussavi-Harami[2,4,5]; Michael Regnier[1,2];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Center for Translational Muscle Research, Seattle, WA, USA
[3]University of Washington, Biology, Seattle, WA, USA
[4]University of Washington, Laboratory Medicine & Pathology, Seattle, WA, USA
[5]University of Washington, Division of Cardiology, Seattle, WA, USA

Sarcomeric proteins function in concert to power muscle contraction, but mutations to individual components can cause organ-scale dysfunction, such as hypertrophic and dilated cardiomyopathies. Here we consider troponin, which regulates the formation of actomyosin cross-bridges via a calcium-mediated shape change that leads to thin filament activation. We have used molecular dynamics simulations to elucidate how troponin's structure changes for a series of mutations in troponin C implicated in hypertrophic and dilated cardiomyopathies. By simulating all three subunits of the troponin complex, we retain the connected structure of the protein, allowing the impact of a point mutation to propagate throughout the protein. These models underscore that impairment in calcium binding and alterations in TnI's interaction with the hydrophobic patch of TnC lead to dysfunction. Further, we are deploying steered molecular dynamics to inform how mutations in the N lobe of cardiac troponin C alter calcium affinity and the interaction of the switch peptide with the hydrophobic patch. Ultimately, these steered molecular dynamics results can inform a spatially explicit model of the half sarcomere to predict how twitches change when troponin is mutated, bridging between the molecular scale and the emergent behavior of the half sarcomere.

WALKING A THIN (FILAMENT) LINE: INTEGRATING TIME-RESOLVED FRET AND COMPUTATION TO IDENTIFY NOVEL PATHOGENIC MECHANISMS IN HYPERTROPHIC CARDIOMYOPATHY

Jil C. Tardiff;
[1]University of Arizona, Biomedical Engineering, Tucson, AZ, USA

One of the most common and earliest clinical manifestations of hypertrophic cardiomyopathy (HCM) is a failure of the heart to efficiently relax in the context of an increase in hemodynamic demand. While in later stages of disease progression the mechanism for this impaired diastolic performance is multifactorial, the primary role of the cardiac thin filament (cTF) in the molecular initiation of relaxation via alterations in calcium exchange kinetics suggests a potential primary "trigger" for early onset disease. The physical interface between the N-lobe of TnC (Site II - calcium binding/release) and the N-terminus of cTnI (Ser23/24 PKA-substrate site) is a complex and dynamic domain that is intricately involved in the acceleration of calcium dissociation rate, tuning diastolic performance to meet hemodynamic demand. The structural mechanism for this observation not been studied in reconstituted cTF in-vitro systems, limiting our understanding of biologic and pathogenic HCM mechanisms. We hypothesize that N-cTnI contributes to stabilizing the calcium atom in Site II via direct interactions with cTnC and that phosphorylation of Ser 23/24 alters these interactions allowing for an increase in calcium dissociation rate. To address this, we performed TR-FRET experiments between cTnI-A28C to cTnC-84C, cTnI-A17C to cTnC-84C, and cTnI-A9C to cTnC-84C in cTF to map N-cTnI with respect to cTnC under different biochemical conditions and in the presence of cTnT HCM mutations. We found that N-cTnI is more disordered in the absence of calcium and become more ordered and closer to cTnC upon the addition of calcium. Phosphorylation of Serine 23/24 caused an increase in distance and FWHM for the A28C and A17C sites, supporting an increase in calcium dissociation rate. MD simulations were employed to determine both average structures and predicted interactions. We predict that this central mechanism is disrupted in a subset of HCM mutations, representing a potential novel therapeutic target.

COMPARISON OF THE CARDIAC TROPONIN CONFORMATION DETERMINED BY POLARIZED FLUORESCENCE AND CRYO-EM

Ivanka R Sevrieva[1]; Andrea C Knowles[1]; Thomas Kampourakis[1]; Yin-Biao Sun[1]; Malcolm Irving[1];
[1]King's College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom

Recent advances in cryo-electron microscopy (cryo-EM) led to 7Å-resolution structural models of the cardiac troponin core domain in isolated thin filaments at different calcium concentrations (Yamada et al. Nat Comm 2020; Risi et al. PNAS 2021) and a better understanding of the changes in thin filament structure associated with calcium binding. However, these structural models may not fully reproduce native thin filament structure in the intact lattice of thin and thick filaments in a muscle cell. Polarized fluorescence from sets of bifunctional probes attached to specific helices in a target protein provides a complementary approach that can be applied in native intracellular conditions, and we previously used that approach to determine the in situ orientations of the N- and C-terminal lobes of TnC during relaxation and active contraction of cardiac muscle cells (Sevrieva et al. JMCC 2014). The C-lobe of cTnC is part of a well-defined domain of troponin called the 'IT arm', and our results showed that the long axis of the IT arm is at about 65° to the filament axis independently of calcium concentration [Ca^{2+}]. The orientation of the IT arm in the cryo-EM models at high [Ca^{2+}] is almost identical to that determined in situ by polarised fluorescence, but that at low [Ca^{2+}] is slightly more parallel to the filament axis. However, in contrast with the cryo-EM models, the in situ method shows that the N-lobe of cTnC does not take up a single conformation on the thin filament at either low or high [Ca^{2+}], suggesting that it is in dynamic equilibrium between multiple conformations. In this respect, the cardiac thin filament is distinct from that of skeletal muscle, in which the N-lobe of sTnC does take up a well-defined conformation at both low and high [Ca^{2+}].

IMPROVING SMALL-MOLECULE UPTAKE USING SIMULATIONS AND DATA TROPONIN-I INDUCED TROPOMYOSIN PIVOTING DEFINES CARDIAC THIN FILAMENT STRUCTURE IN RELAXED MUSCLE.

William Lehman[1]; Balajee Ramachandran[1]; Michael Rynkiewicz[1];
[1]Boston University Chobanian & Avedisian School of Medicine, Department of Physiology & Biophysics, Boston, MA, USA

By occupying one of three meta-stable configurations, tropomyosin regulates access of myosin motor-heads to their actin-binding sites and thus the crossbridge cycle that drives contraction. At low-calcium concentrations, tropomyosin is trapped by troponin-I in an inhibitory B-state that sterically blocks myosin-binding to actin and leads to muscle relaxation. Calcium-binding to TnC draws TnI away from tropomyosin, while tropomyosin itself moves to a C-state location over actin. This partially relieves the steric-inhibition and allows weak-binding of myosin-heads to actin. Tropomyosin is thought to oscillate about the C-state position while myosin-heads transition to strong actin-bound configurations, fully-activating the thin filament. Nevertheless, the structural reconfiguration of thin filaments that accompanies the calcium-sensitive B-state/C-state shift in troponin/tropomyosin on actin remains uncertain and at best is described by moderate-resolution cryo-EM reconstructions. Our recent computational studies indicate that intermolecular residue-to-residue salt-bridge formation between actin and tropomyosin is indistinguishable in B- and C-state thin filament configurations. We ask, how then is it possible for tropomyosin to reposition, let-alone slide or roll over actin between B- and C-states, as variously proposed? Using molecular modeling and molecular dynamics simulations, we show instead that tropomyosin pivots about relatively fixed points on actin, and that this pivoting accounts for the B- to C-state change in the average center-of-mass of tropomyosin on actin. We argue that at low-calcium concentration, C-terminal domains of TnI attract the tropomyosin coiled coil which bends toward TnI to block myosin-binding, while still maintaining contact with its underlying actin-binding sites. Measurement of interaction energetics suggests that muscle activation and ensuing C-terminal TnI detachment from actin-tropomyosin involves tropomyosin pivoting back to an energetically-favorable C-state orientation.

STRUCTURES AND MECHANISMS OF ACTIN ATP HYDROLYSIS

Yuichiro Maeda[3,6,12]; Yusuke Kanematsu[1,2]; Akihiro Narita[3,4]; Toshiro Oda[5]; Ryotaro Koike[6]; Motonori Ota[6]; Yu Takano[1]; Kei Moritsugu[7]; Ikuko Fujiwara[8]; Kotaro Tanaka[3]; Hideyuki Komatsu[9]; Takayuki Nagae[10]; Nobuhisa Watanabe[10]; Mitsusada Iwasa[6]; Shuichi Takeda[3,11];
[1]Hiroshima City University, Graduate School of Information Sciences, Hiroshima, Japan
[2]Hiroshima University, Graduate School of Advanced Science and Engineering, Hiroshima, Japan
[3]Nagoya University, Structural Biology Research Center, Nagoya, Japan
[4]Nagoya University, Graduate School of Science, Nagoya, Japan
[5]Tokai Gakuin University, Faculty of Health and Welfare, Kakamigahara, Japan
[6]Nagoya University, Graduate School of Informatics, Nagoya , Japan
[7]Yokohama City University, Graduate School of Medical Life Science, Yokohama, Japan
[8]Osaka City University, Graduate School of Science, Osaka, Japan
[9]Kyushu Institute of Technology, Graduate School of Computer Science and System Engineering, Iizuka, Japan
[10]Nagoya University, Synchrotron Radiation Research Center, Nagoya, Japan
[11]Okayama University, Research Institute for Interdisciplinary Science, Okayama, Japan
[12]Toyota Physical and Chemical Research Institute, Nagakute, Japan

The major cytoskeleton protein actin undergoes cyclic transitions between the monomeric G-form and the filamentous F-form, which drive organelle transport and cell motility. This mechanical work is driven by the ATPase activity at the catalytic site in the F-form. For deeper understanding of the actin cellular functions, the reaction mechanism must be elucidated. Here, we show that a single actin molecule is trapped in the F-form by fragmin domain-1 binding and present their crystal structures in the ATP analog-, ADP-Pi-, and ADP-bound forms, at 1.15-Å resolutions. By applying quantum mechanics/molecular mechanics calculations to the structures, we have revealed a consistent and comprehensive reaction path of ATP hydrolysis by the F-form actin. The reaction path consists of four steps: 1) W1 and W2 rotations; 2) P^G–O^{3B} bond cleavage; 3) four concomitant events: W1–PO_3^- formation, OH^- and proton cleavage, nucleophilic attack by the OH^- against P^G, and the abstracted proton transfer; and 4) proton relocation that stabilizes the ADP-Pi–bound F-form actin. The mechanism explains how the G-to-F conformational transition triggers ATP hydrolysis, the slow rate of ATP hydrolysis by actin, and the irreversibility of the hydrolysis reaction. Remarkably, while the actin active site structure is much simpler than those of some motor proteins, like myosin, actin and the motor proteins share the common reaction path. This common mechanism is now robust, since with actin both the initial and the final structures of the ATP hydrolysis reaction were available at high resolutions for the first time among ATPase proteins. This is made possible due to a unique property of actin; with actin, the end of ATP hydrolysis is not associated with a global protein conformational change. This makes the ADP-Pi-bound state stable. With actin, Pi-release could be due to structural fluctuations, not due to a global conformational change.

THE UNSTRUCTURED C-TERMINAL REGION OF TROPONIN T RETARDS CALCIUM ACTIVATION OF STRIATED MUSCLE

Joseph M Chalovich[1]; Li Zhu[1];
[1]Brody School of Medicine ECU, Biochem & Mol Biol, Greenville, NC 27834, NC, USA

Calcium binding to TnC alters contacts among troponin subunits leading to reorientation of tropomyosin on actin and a large increase in activity. Eisenberg and Weihing (10.1038/2281092a0) were the first of many to show that myosin lacking bound ATP or ADP-Pi (activating states) can further increase activity even at saturating calcium. That is, something in the thin filament retards full calcium activation. That limitation of activation requires basic residues within the C-terminal 14-16 residues of TnT. Removal of those residues from TnT doubles the ATPase activity. Negative charges introduced at some positions in that region of TnT are also activating. Fluorescence and kinetics measurements show that the unstructured C-terminal basic region of TnT stabilizes the inactive state of regulated actin formed in the absence of calcium and "activating" species of myosin (see 10.1016/S0006-3495(81)84777-7 for activating species) and destabilizes the active state at high calcium levels. Furthermore, the C-terminal basic region of TnT increases the calcium level required for activation of skeletal (10.1021/acs.biochem.0c00499) and cardiac (10.1021/acs.biochem.0c00430) fibers. A FRET analysis showed that calcium binding positioned the C-terminal region of TnT, and the inhibitory and switch regions of TnI away from actin-tropomyosin (10.1021/acs.biochem.2c00090). Unlike those regions of TnI, the C-terminal region of TnT did not appear to contact TnC, although it moved toward it. Removal of the charged residues from C-terminal TnT mimicked the calcium induced changes in the position of C-terminal TnT and the inhibitory region of TnI. However, the switch region of TnI was not near TnC as it was in the calcium state. Conclusion: The disordered, basic, C-terminal region of TnT is necessary for full inactivation, it inhibits activation by Ca^{2+} and permits full activation upon binding activating forms of myosin to actin. It does all of this in concert with the other known elements of troponin.

MODEL BUILDING CHALLENGES AND THE TIME-SCALE PROBLEM IN THE COMPUTATIONAL STUDY OF MUSCLE BIOPHYSICS.

Steven D. Schwartz;
[1]The University of Arizona, Chemistry and Biochemistry, Tucson, AZ, USA

The molecular machines of muscle present some standard and some unique challenges to theoretical investigation. The first challenge is encountered when there is no extant atomic structure. We faced this years ago in the cardiac thin filament when only fragments and the troponin core had any published structures. Docking and molecular minimization allowed us to create models of fully solvated thin filament. New cryo-em structures will help to alleviate such problems, but this technology is unable to find average structures and range of motion for mobile regions. In this case our collaboration with Jil Tardiff's group has proved transformative. Given a structure, however; the challenges of developing usable computational approaches remain. The number of atoms in a fully solvated model of just the cardiac thin filament is in the multiple millions. Because an atomistic simulation is required to accurately evolve the state for the fastest timescale in the system accessible times are limited to nanosecond to microsecond times even on the fastest GPU accelerated processors – clearly far less than any biologic timescale. Our group has developed and applied enhanced sampling methods to overcome the timescale problem – examples of this are transition path sampling for reactions such as the hydrolysis of ATP by myosin and metadynamics for conformational transitions. We will present such methods and results. A final approach we have employed is to use short time simulations (nanosecond) to compare wildtype to mutant thin filament complexes. This is done with the understanding that the overall structure of the thin filament must be stable. Thus, relatively short timescale simulations are expected to allow inference of the impact of mutation. Lately we have coupled this approach with machine learning algorithms to make predictions of pathogenicity of variants of unknown significance.

MULTISCALE REGULATION OF SARCOMERES IN HEALTH AND DISEASE

Leslie A. Leinwand[1];
[1]BioFrontiers Institute at the University of Colorado, Boulder, Boulder, CO, USA

Muscle sarcomeres are complex mixtures of many interconnecting proteins. Our interests have long focused on the myosin motor protein family and how mutations in its members cause a variety of cardiac and skeletal muscle myopathies. With the knowledge gained from decades of study of these motor molecules and mutations in them, it has been possible to develop the first FDA-approved therapy for the most common inherited cardiomyopathy, hypertrophic cardiomyopathy. In this talk, I will describe the rationale for inhibiting MYH7 (AKA β-myosin) and how this same approach might be useful in treating a skeletal muscle myopathy caused by mutations in MYH7. One understudied area of muscle is driven by the fact that our cardiac muscle cells are as old as we are, but the contents of their sarcomeres and the rest of the proteomes need to be repaired and replaced. How are those processes regulated and how are they affected by different diseases, including myosin-based myopathies? We are using several approaches to study protein homeostasis in health and disease. We are pairing stable isotope labeling in wild type and genetic mouse models followed by mass spectrometry and imaging to generate spatial information about ages of sarcomeres, and individual protein by bulk protein half life determination.

WHAT DO MODELERS NEED FROM EXPERIMENTALISTS AND VICE VERSA?

Malcolm Irving
Kings College London, United Kingdom

No Abstract

PREDICTING CLINICAL PHENOTYPES OF TPM1 MISSENSE MUTATIONS

Stuart G. Campbell[1]; Saiti Halder[1]; Michael J Rynkiewicz[2]; Jeffrey R Moore[3]; William Lehman[2];
[1]Yale University, Biomedical Engineering, New Haven, CT, USA
[2]Boston University Medical Center, Pharmacology, Physiology, & Biophysics, Boston, MA, USA
[3]University of Massachusetts Lowell, Biological Science, Lowell, MA, USA

Mutations to the gene encoding human cardiac tropomyosin (TPM1) have been linked with a range of clinically significant inherited cardiomyopathies. Although TPM1 genetic variants can be readily identified through gene sequencing, the mere presence of such a variant is not deemed actionable for the treatment of a patient unless or until additional information on the pathogenicity of the variant can be obtained. Because cost or other factors make it impossible to generate a mouse model or perform linkage analysis for each new TPM1 variant, we have sought a scalable computational approach to evaluate the physiological consequences (and hence pathogenicity) of arbitrary TPM1 missense variants. Our computational pipeline brings together atomistic simulations of the cardiac thin filament, coarse-grain models of tropomyosin bending, and Markov-type models of thin filament regulatory dynamics to make predictions of mutation effects. Simulations of steady-state regulated in vitro motility and isometric twitch dynamics are then validated experimentally. The degree to which quantitative multi-scale predictions are possible is demonstrated through a series of specific cases, including clinically well-characterized mutations and attempts to screen several TPM1 variants of unknown significance.

FSGE: A COMPUTATIONAL MODEL FOR EQUILIBRATED CARDIOVASCULAR FLUID-SOLID-GROWTH INTERACTION

Martin R Pfaller[1,2,3]; Marcos Latorre[4]; Erica L Schwarz[6]; Fannie M Gerosa[1,3]; Jason M Szafron[1]; Jay D Humphrey[5]; Alison L Marsden[1,2,3];
[1]Stanford University, Pediatrics, Stanford, CA, USA
[2]Stanford University, Maternal & Child Health Research Institute, Stanford, CA, USA
[3]Stanford University, Institute for Computational and Mathematical Engineering, Stanford, CA, USA
[4]Universitat Politècnica de València, Center for Research and Innovation in Bioengineering, Valencia, Spain
[5]Yale School of Medicine, Vascular Biology and Therapeutics Program, New Haven, CT, USA
[6]Stanford University, Bioengineering, Stanford, CA, USA

Growth and remodeling (G&R) are commonly triggered mechanically through fluid-structure interaction by a combination of pressure-induced intramural stress and flow-induced wall shear stress. Previous G&R models rely on simplifying assumptions for fluid dynamics of blood inside the vessel. We propose to combine fluid-structure interaction and G&R in a novel fluid-solid-growth (FSGe) framework using a mechanobiologically equilibrated version of the constrained mixture theory. We solve the incompressible Navier-Stokes equations to obtain velocity and pressure inside the blood vessel. At the fluid-solid interface, we pass local fluid pressure and wall shear stress to our large-deformation solid G&R model. As the solid model, we use a fast and efficient formulation of the constrained mixture theory, assuming that each G&R state is mechanobiologically equilibrated. A shear-to-intramural gain ratio controls the importance of wall shear stress and intramural stress stimuli. Both fields are coupled in a partitioned scheme, separating fluid and solid time scales (seconds vs. weeks). The computational cost of our coupling scheme is comparable to a fluid-structure-interaction simulation with a hyperelastic solid material. We compare our coupled FSGe method to G&R models with different fluid approximations for pressure and wall shear stress. Our examples include aneurysmal formation, stenosis formation, and distortions of blood flow for various Reynolds numbers. Simplified fluid dynamics in cardiovascular G&R models can provide a good approximation in many scenarios. However, we show that local fluid dynamics can significantly influence the long-term G&R state in blood vessels. This work was supported by NIH Grants K99HL161313, R01HL139796, R01HL159954, the Additional Ventures Foundation Cures Collaborative, and the Stanford Maternal and Child Health Research Institute.

EXPERIMENTAL AND COMPUTATIONAL APPROACHES FOR MECHANISTIC ANALYSIS AND THERAPEUTIC DEVELOPMENT OF 2-DEOXY ATP TO TREAT HEART FAILURE

Michael Regnier[1]; Charles Murry[2]; Ketaki Mhatre[3];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Pathology & Lab Medicine, Seattle, WA, USA
[3]University of Washington, Heart and Muscle Mechanics Lab, Seattle, WA, USA

2-deoxy-ATP (dATP) is a naturally occurring myosin activator that improves contractility without impairing relaxation. ATP is converted to dATP by the enzyme Ribonucleotide Reductase (RNR). In post-mitotic cardiomyocytes RNR is downregulated but over-expression of the enzyme results in elevation from < 0.1% to ~1% of the ATP pool. In transgenic mice that have 1% dATP, left ventricular (LV) pressure development is increased by ~30% and the rate of pressure development (+dP/dT) and decline (-dP/dT) are increased. We have used contractile mechanics and protein biochemistry to determine that dATP increases crossbridge cycling rate and NTPase activity, speeding the rate of myosin attachment to actin, and the release of NTP hydrolysis products, and that contractile force is augmented with as little as 1-2% dATP. We used x-ray diffraction of demembranated pig LV muscle to demonstrate that this level of dATP results in significant movement of myosin heads away from the thick filament and towards thin filaments. Stiffness measurements indicate weak binding with actin is also increased. We used Molecular Dynamics (MD) modeling to demonstrate this is likely due to a dADP.Pi induced increase in positive charge of the actin binding surface on myosin. MD simulations were also used to demonstrate that M.ADP.Pi may destabilize the interacting heads motif (IHM) of myosin on the thick filament backbone, providing a mechanism for increased myosin recruitment during contractions. Multi-scale modeling predicts that the increased recruitment and cycling rate of myosin can explain the increases in LV hemodynamics. We recently demonstrated that human stem cell-derived cardiomyocytes, engineered to over-express RNR, result in 1-2% dATP in the cells. When these cells are transplanted into rats at one-month post-myocardial infarct, they couple with host myocardium and deliver dATP through gap junctions. This results in improved LV function for at least three months post-transplant.

MODULATING FAST SKELETAL MUSCLE CONTRACTION AS A NOVEL STRATEGY FOR THE PROTECTION OF SKELETAL MUSCLE IN MUSCULAR DYSTROPHY

Alan J. Russell; Susan Brooks[3]; Dennis Claflin[3]; H Lee Sweeney[2]; Beth Barton[2]; Peter Nguyen[4]; Alexis Rutledge[4]; Mike DuVall[1]; Ben Barthel[1]; Angela K Peter[1]; Molly Madden[1]; Breanne Newell-Stamper[1]; Kevin Koch[1];
[1]Edgewise Therapeutics, Boulder, CO, USA
[2]University of Florida, Department of Applied Physiology and Kinesiology and Myology Institute, Gainesville, FL, USA
[3]University of Michigan, Department of Surgery, An Arbor, MI, USA
[4]Texas A&M University, Department of Veterinary Integrative Biosciences, College Station, TX, USA
[5]University of Colorado, Boulder, Department of Molecular, Cellular, and Developmental Biology and BioFrontiers Institute, Boulder, CO, USA

Duchenne muscular dystrophy (DMD) is a lethal muscle disease caused by absence of the protein dystrophin, which acts as a structural link between the basal lamina and contractile machinery to stabilize muscle membranes from mechanical stress. In DMD, mechanical stress leads to exaggerated membrane injury and fiber breakdown, with fast fibers being the most susceptible to damage. A major contributor to this injury is muscle contraction, controlled by the motor protein myosin. However, the relationship between how muscle contraction and fast muscle fiber damage contribute to the pathophysiology of DMD has not been well characterized. We explored the role of fast skeletal muscle contraction in DMD with a novel, selective, orally active inhibitor of fast skeletal muscle myosin, EDG-5506. Surprisingly, even modest decreases of contraction (<15%) were sufficient to protect skeletal muscles in dystrophic mdx mice from stress injury. Longer-term treatment also decreased muscle fibrosis in key disease-implicated tissues. Importantly, therapeutic levels of myosin inhibition with EDG-5506 did not detrimentally affect strength or coordination. Finally, in dystrophic dogs, EDG-5506 reversibly reduced circulating muscle injury biomarkers and increased habitual activity. This unexpected biology may represent an important alternative treatment strategy for Duchenne and related myopathies.

PRECLINICAL CHARACTERIZATION OF CK-4021586, A NEW CLASS OF CARDIAC MYOSIN INHIBITORS FOR THE TREATMENT OF HYPERTROPHIC CARDIOMYOPATHY

James J Hartman[1]; Saswata S Sarkar[1]; Darren T Hwee[1]; Chihyuan Chuang[1]; Peadar Cremin[1]; Andre deRosier[1]; Samantha Edell[1]; Roshni Madhvani[1]; Yangsong Wu[1]; Lisette Yco[1]; Jeanelle Zamora[1]; Anne Murphy[1]; Bradley P Morgan[1]; Fady I Malik[1];
[1]Cytokinetics, Inc., Preclinical Research, South San Francisco, CA, USA

Hypertrophic cardiomyopathy (HCM) is the most common monogenetic heart disease, with an estimated carrier prevalence of ~1 in 500. Hypercontractility of the cardiac sarcomere is a common driver of the pathological hypertrophic cardiac remodeling that is a hallmark of the disease. Clinical trials of the selective cardiac myosin inhibitors mavacamten and aficamten have demonstrated that inhibition of the biochemical activity of cardiac myosin can induce cardiac remodeling and improve both patient symptoms and exercise capacity in HCM with left ventricular outflow tract obstruction (oHCM). The diverse genetic underpinnings of HCM suggest that it is possible that additional classes of cardiac myosin inhibitors with distinct biochemical mechanisms-of-action may also have a salutary impact on disease expression. While both mavacamten and aficamten bind to the single-headed motor domain of myosin (subfragment-1) and inhibit its ATPase activity, CK-4021586 (CK-586) is a new class of cardiac myosin inhibitor that inhibits the ATPase activity of two-headed heavy meromyosin (HMM) but not single-headed subfragment-1. CK-586 is a partial inhibitor of cardiac myofibrillar ATPase activity (EC50 2.9 μM, maximal inhibition ~50%) that requires the regulatory light chain. Notably, fractional shortening of electrically-paced adult rat ventricular cardiomyocytes was inhibited almost completely (>80% at 5 μM) without alterations in the calcium (Ca2+) transient. In normal Sprague Dawley rats, CK-586 reduced cardiac fractional shortening in a dose and concentration-dependent manner. In conclusion, CK-586 is a novel, small molecule, cardiac myosin inhibitor that reduces cardiac contractility in vitro and in vivo. CK-586 has a biochemical mechanism of action distinct from both mavacamten and aficamten, providing an additional tool to decrease the number of functionally-available myosin heads and treat the cardiac hypercontractility that forms the pathologic basis of HCM.

DRUG DESIGN TARGETING MYOSIN BLEBBISTATIN BINDING SITE

Andras Malnasi-Csizmadia[1]; Anna Rauscher[2]; Mate Gyimesi[1];
[1]Eötvös University, Dept. of Biochemistry, Budapest, Hungary
[2]Motorpharma Ltd, Budapest, Hungary

Since the discovery of the first myosin2 specific inhibitor, blebbistatin, several research groups have designed and synthesised thousands of compounds that bind to the blebbistatin binding site. These compounds are derivatives of several different chemical skeletons and have very different biochemical and pharmacological properties. In the meantime, two of these compounds are already in clinical trials for a variety of indications. In my talk I will summarise the historical aspects of these investigations and the recent progress in the field.

MULTISCALE SIMULATIONS OF THE EFFECTS OF 2'-DEOXY-ATP AND MYOSIN MUTATIONS ON ACTOMYOSIN INTERACTIONS

Marcus Hock[1]; Matthew C Childers[3]; Abigail Teitgen[1]; Gary Huber[2]; J. Andrew McCammon[2]; Michael Regnier[3]; Andrew D McCulloch[1];
[1]University of California San Diego, Bioengineering, La Jolla, CA, USA
[2]University of California San Diego, Chemistry and Biochemistry, La Jolla, CA, USA
[3]University of Washington, Bioengineering, Seattle, WA, USA

2'-deoxy-ATP (dATP) is a myosin activator, which increases contractile force in cardiomyocytes even at low abundances in the nucleotide pool. dATP has therefore been proposed as a potential therapeutic for heart failure with reduced ejection fraction. Here, we use a novel combined molecular and Markov-Brownian dynamics model to investigate changes in acto-myosin dynamics due to dATP compared with ATP. As possible treatment targets, we also simulated two dilated cardiomyopathy causing mutations in β-myosin heavy chain myosin A223T and S532P, which have measured changes in cross-bridge kinetics and therefore expected changes in acto-myosin association. Molecular dynamics (MD) simulations of pre-powerstroke myosin were performed in four conditions: Wild Type (WT), A223T, and S532P myosin in the presence of ADP.Pi, and WT in the presence of dADP.Pi. The MD trajectories were clustered into microstates, before being coarse grained into a reduced Markov model consisting of 15 meta-stable conformations, per each condition. By using multiple different metastable conformations from MD trajectories as inputs into the BD simulations, we expect these simulations will provide greater insights into protein-protein association kinetics. Furthermore, we have implemented geometric restraints into the Brownian dynamics simulations to better reflect the tethered natured of the myosin head to the thick filament. Analysis of the Markov models revealed that dADP.Pi reduces conformational variability of myosin head. Neither the A223T nor S532P mutations affected the rates of transitions between conformations. BD simulations showed that conformational variability leads to significant differences in association rates of myosin to actin, producing up to a threefold change between different conformations from a single MD trajectory. Overall comparisons between conditions demonstrate that dADP.Pi increases actin binding affinity of WT myosin by approximately twofold. The A223T + ADP.Pi and S532P + ADP.Pi conditions showed a 0.56x and 3.0x change in association relative to WT myosin, respectively.

STRUCTURAL DYNAMICS OF THE THICK FILAMENT DURING THE PHYSIOLOGICAL CARDIAC CYCLE.

Yanhong Wang[1]; Luca Fusi[1]; Jesus G Ovejero[1]; Cameron Hill[1]; Samina Juma[1]; Flair Paradine Cullup[1]; So-Jin Park-Holohan[1]; Theyencheri Narayanan[2]; Malcolm Irving[1]; **Elisabetta Brunello**[1];
[1]King's College London , London, United Kingdom
[2]European Synchrotron Radiation Facility, Grenoble, France

The contraction of heart muscle is regulated by a dual-filament mechanism in which calcium-dependent structural changes in the actin-containing thin filaments control the number of accessible binding sites for the myosin motors, while regulatory changes in the myosin-containing thick filaments modulate the number of available motors and the kinetics of force development and relaxation. Here we investigated the structural dynamics of the thick filament in electrically-paced cardiac trabeculae isolated from rat hearts using a combination of time-resolved small-angle X-ray diffraction and mechanical protocols that reproduce the four phases of the physiological cardiac cycle: isometric contraction, ejection, isometric relaxation and refilling. Sarcomere length was maintained at the diastolic value during active force development, shortened by 12% while force was held at ~50 kPa, was held constant during the first phase of relaxation, before being re-stretched to the initial value at the end of mechanical relaxation. The force-sarcomere length relationship in this protocol mimics the pressure-volume relationship in the intact heart, and the associated regulatory structural changes in the thick filament and myosin motors represent a first step towards understanding the role of myosin-based regulation in the control of the physiological cardiac cycle. Supported by BHF, Wellcome Trust (UK), and ESRF (France).

CUT AND PASTE OF MYOSIN BINDING PROTEIN-C IN SKELETAL MUSCLES

Samantha P Harris[1]; Nichlas M Engels[1]; Rachel L Sadler[1]; Michel Kuehn[2]; Devin Nissen[3]; Weikang Ma[3]; Thomas Irving[3]; Wolfgang A Linke[2]; Anthony L Hessel[2];
[1]University of Arizona, Physiology, Tucson, AZ, USA
[2]University of Muenster, Institute of Physiology II, Muenster, Germany
[3]Illinois Institute of Technology, BioCat, Department of Biology, Chicago, IL, USA

The family of proteins known as myosin binding protein-C (MyBP-C) are regulatory proteins localized to muscle sarcomeres that are encoded by 3 separate genes (MYBPC1, MYBPC2, and MYBPC3). The 3 genes encode distinct MyBP-C protein paralogs expressed in fast skeletal, slow skeletal, and cardiac muscles, respectively. However, due to co-expression of the two skeletal paralogs (and their related multiple splice variants) it has been challenging to describe distinct functional roles for each MyBP-C because skeletal muscles typically express a mixture of fiber types. Here, we overcome this challenge by engineering two new mouse models that selectively target MYBPC1 and MYBPC2 to permit removal and replacement (cut and paste) of each protein paralog in permeabilized muscle fibers in situ. The objective of this study was to determine functional and structural effects of selective loss of fast skeletal and slow skeletal MyBP-C in predominantly slow twitch (soleus) and fast twitch (psoas), muscle respectively. Results of force measurements in soleus and psoas showed that tension-pCa relationships were right-shifted following treatment with TEVp, indicating reduced Ca^{2+} sensitivity of tension following selective loss of either sMyBP-C or fMyBP-C. Notably, spontaneous contractile oscillations (SPOC) were also frequently observed following loss of MyBP-C. X-ray diffraction experiments in permeabilized psoas fibers showed significant changes in sarcomere structure including changes in lattice spacing, $I_{1,1}/I_{1,0}$ ratios, and reflections attributable to both thick and thin filament structures (e.g., M3 and M6 spacing and A6 and A7 spacing, respectively). Conclusions: We interpret these data in terms of a model where the skeletal muscle paralogs of MyBP-C exert both structural and functional effects at rest and in contracting muscle, potentially by binding to both thick and thin filaments simultaneously.

UNDERSTANDING HYPERTROPHIC CARDIOMYOPATHY (HCM) ACROSS THE THICK AND THIN FILAMENT, BRIDGING IN-VITRO AND IN-SILICO MODELS OF DISEASE TO ACCELERATE PATHOMECHANISM DISCOVERY

Christopher N. Toepfer[1];
[1]University of Oxford, Oxford, United Kingdom

Approximately 1 in 500 people inherit a gene that can cause hypertrophic cardiomyopathy, a disease that is typified by increased incidences of atrial fibrillation, heart failure, and sudden cardiac death. There are many genetic variants across a variety of disease-causing genes that drive HCM, and the disease driving pathomechanisms are not fully understood. Many of the genes involved in HCM are encoding the proteins that drive or regulate muscle contraction in the cardiac sarcomere. Some of these genes are in the proteins of the thick filament including myosin and myosin binding protein-C, which drive and adapt muscle contraction. Other variants are those of the thin filament, which regulate contraction altering myofilament sensing of calcium. We employ in-vitro iPSC-CM models twinned with CRISPR/Cas-9 to model functional changes of human cardiomyocytes in the dish. We then use this in-vitro data to model HCM in-silico by application of biological data to in-silico models of adult cardiomyocytes. This allows us to make predictions of the disease-causing mechanism. Meshing these two techniques has allowed us to understand the key contractile parameters that may best be targeted to help treat disease and define druggable targets within the cardiac sarcomere. With the aim of developing novel genetically targeted therapies in HCM.

MYOSIN BASED REGULATION: THE PLOT THICKENS

Thomas C Irving[1];
[1]Illinois Institute of Technology, Biology, Chicago, IL, USA

There is a growing appreciation that thick filament-based regulation mechanisms act in parallel to the textbook thin filament regulation in striated muscle. Evidence suggesting this comes primarily from 1) biochemical assays showing that, under relaxed conditions, myosin is found either in a disordered-relaxed (DRX) state that are readily for contraction or in a very low ATP consumption, super-relaxed (SRX) state that is unavailable for actin binding and 2) structural assays showing that, in a resting muscle, a large percentage of myosin heads are found in a quasi-helically ordered OFF state held close to the thick filament backbone where this helical ordering is lost when myosin heads are turned ON to participate in contraction. It is widely assumed that myosin heads in the biochemically-defined SRX state are strictly equivalent to the structurally-defined OFF state and heads in the DRX state are strictly equivalent to ON state heads so that SRX and the OFF state can be been used interchangeably. This view now appears to be an over-simplification. Here I discuss the results of small angle X-ray diffraction and ATP turnover studies with various inotropic interventions and tool compounds that demonstrate conditions where SRX/DRX transitions are well correlated with the structural OFF-ON transitions but, importantly, multiple situations where this correlation breaks down. I suggest that the biochemically defined term SRX should be reserved to refer to only the very low ATP consumption state that may or may not be ordered. The structurally defined OFF state should be reserved only to refer to quasi-helically ordered heads closely associated with the thick filament backbone that may or may not be in the SRX. There may also be multiple populations of disordered (DRX) and ordered heads that may or may not be available to interact with actin.

SINGLE MOLECULE IMAGING APPROACHES TOWARDS UNDERSTANDING DUAL FILAMENT REGULATION IN MUSCLE

Neil Kad
University of Kent, United Kingdom

No Abstract

RAMP-STRETCHES DURING RELAXATION OF TWITCHING INTACT CARDIAC TRABECULA SUGGEST A NEED FOR DYNAMIC MODELS OF MYOFILAMENT FUNCTION

Bertrand C Tanner[1]; Bradley M Palmer[2]; **Charles S Chung**[3];
[1]Washington State University, Integrative Physiology and Neuroscience, Pullman, WA, USA
[2]University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA
[3]Wayne State University, Physiology, Detroit, MI, USA

We have previously found that relaxation is dependent on the strain rate of a lengthening stretch just prior to relaxation, also referred to as Mechanical Control of Relaxation. To investigate the mechanisms underlying Mechanical Control of Relaxation, we sought to characterize experimentally induced ramp-stretches using existing models of stress-responses to muscle stretch. Ramp-stretches of varying strain rates (amplitude=1% muscle length) were applied to intact rat cardiac trabeculae following a load-clamp at 50% of the maximal developed twitch force, which provide a first-order estimate of ejection and coupling to an afterload. The resultant stress-response was calculated as the difference between the time-dependent stress profile between load-clamped twitches with and without a ramp-stretch. The stress-response exhibited features of the step-stretch response of activated, permeabilized myocardium, such as distortion-dependent peak stress, rapid force decay related to crossbridge detachment, and stress recovery related to crossbridge recruitment. The peak stress was strain rate dependent, but the minimum stress and the time-to-minimum stress values were not. As the stretches occurred later into diastole, a more passive stress-response was observed. Three mathematical models with parameters representing crossbridge attachment and detachment kinetics were fit to the stretch responses to assess whether crossbridge kinetics showed a strain rate-dependence, as predicted by prior studies (Kawai and Brandt 1980, Palmer et al 2007, Palmer et al 2020). An explicit strain-dependence in the kinematic model suggests that crossbridge detachment rates increase as strain rates increase, but all models showed some limitation the fits, especially near the nadir of the stress response. A substantive limitation of these models is likely the assumption of constant calcium and thin filament activation. Additionally, models including more explicit (fewer lumped) parameters and/or time-varying changes (Smith and Geeves 1995, Campbell 2014) may provide improved mechanistic insight into strain-rate dependent changes of myofilaments during relaxation.

ARE THE DRX AND SRX STATES IN THERMAL EQUILIBRIUM. A PARADOX?

Michael A Geeves[1]; Max Mahoney-Schaefer[2]; Kristina Kooiker[2]; Saffie Mohran[2]; Jeremy Freeman[2]; Michael Regnier[2]; Farid Moussavi-Harami[2];
[1]University of Kent, School of Biosciences, Canterbury, United Kingdom
[2]University of Washington, Division of Cardiology & Bioengineering, Seattle, WA, USA

Current views of muscle regulation suggest the presence of a dual regulation system that controls access of myosin cross-bridges to actin sites via both thin and thick filament regulation systems. Evidence for the thick filament regulation comes from structural studies of an order-disorder transition of the thick filament and biochemical assays of the rate of ATP turnover by myosin heads in HMM, myosin, thick filaments myofibrils or cells. Biochemical assays have led to the definition of a super-relaxed state (SRX) which has an ATP turnover rate < $1/10^{th}$ of that of the non-activated myosin (disordered relaxed state, DRX). Both structural and biochemical assays have been interpreted in terms of an interacting head motif (IHM) in which the two heads of a myosin molecule in the M.ADP.Pi state combine to inhibit each other. The ratio of SRX/DRX can be perturbed by temperature, small molecules (mavacamten) and myosin mutations. The problem with the simple ATPase assay of the SRX is that it is very simple to do but without care can be easily misinterpreted. Here we will show some of the problems encountered in assaying the SRX with porcine cardiac HMM & S1, and lessons that can be learnt from the assays that were done on scallop myosin before the SRX was first described. We demonstrate that there is little clear evidence for an SRX state in porcine cardiac S1 or HMM, that the SRX/DRX and order disorder transitions do not appear to correspond and the SRX and DRX states cannot be in a simple thermal equilibrium. Addition of myosin effectors such as mavacamten, danicamtiv, aficamten, omecamtiv mecarbil and deoxyATP do not appear to affect the DRX/SRX ratio for porcine cardiac HMM.

EVIDENCE FOR TWO DISTINCT ACTOMYOSIN CROSS-BRIDGE STIFFNESS FOR HUMAN BETA CARDIAC MYOSIN

Tianbang Wang[1]; Theresia Kraft[1]; Arnab Nayak[1]; **Mamta Amrute-Nayak**;
[1]Medical School Hannover, Molecular and Cell Physiology, Hannover, Germany

Stiffness, i.e., resistance to elastic deformation, is a crucial physical parameter that determines the force-generation and force-sensing ability of the motor proteins, such as cardiac myosin II. During muscle or cardiac contraction, an elastic element within the actomyosin (AM) cross-bridge allows the strain to develop, and the release of this strain then drives the actin filament sliding relative to the myosin or thick filament. Here, we employed a single-molecule optical trapping method to analyze the rigidity of native human ventricular b-cardiac myosin (b-CM). The intermittent interactions between the actin and myosin in the presence of ATP were analyzed using the variance-covariance method (Lewalle et al., 2008), to determine the stiffness of the myosin. Interestingly, we found that the measured stiffness of the b-CM was sensitive to the ATP concentration. With increasing [ATP] the AM cross-bridge stiffness decreased, suggesting that the stiffness was dependent on the nucleotide state as the myosin progressed through the ATPase cycle. The strongly bound AM.ADP state exhibited > two-fold lower stiffness (~0.6 pN/nm) than the AM rigor state (~1.9 pN/nm). We further confirm the high stiffness value as rigor stiffness by employing pyrophosphate (PPi) in our assays. In the presence of PPi, the actin and myosin underwent binding and unbinding events. The bound state, i.e., nucleotide-free state, thus represented the near-rigor AM state with the stiffness of ~ 2 pN/nm. We speculate that more compliant AM.ADP state may be important for the crossbridge response to assisting or resisting load in cardiac myosins and other myosin forms where ADP release is known to be load-sensitive. Our studies revealed previously unexplored aspects of myosin's mechanical property. This information is vital to understand cardiac disorders such as hypertrophic cardiomyopathy linked to point mutations in b-CM components that alter the rigidity of ventricular myosin.

MYOSIN-BINDING PROTEIN C/H: IMPACT ON MUSCLE CONTRACTILITY AND DEVELOPMENT

David Warshaw
University of Vermont, USA

No Abstract

RECENT BIOPHYSICAL APPROACHES TO INVESTIGATE THE COUPLING BETWEEN FORCE-GENERATION AND PHOSPHATE-RELEASE IN MYOSIN

Edward P. Debold
[1]University of Massachusetts, Amherst, Kinesiology, Amherst, MA, USA

Myosin is the molecular motor that generates the force and motion required to contract our muscles and drive a host of intracellular processes. It does so by transducing chemical energy, derived from the hydrolysis of ATP, into mechanical work. Despite extensive study key aspects of the transduction process remain unclear, most notably the nature of the coupling between the force-generating powerstroke and the release of phosphate (P_i) from the active site. We have performed a series of biochemical and biophysical studies aimed at gaining a deeper understanding of this coupling, including determining the relative timing of the powerstroke and P_i-release. Transient kinetic and FRET-based experiments on myosin Va indicate that the powerstroke occurs more than twice as fast as the rate of P_i-release. Single molecule laser trap assays, using both myosin II and myosin Va, demonstrate that the powerstroke is generated rapidly (<2ms) after the formation of a strong actomyosin bond. Indeed, even in construct with a mutation (S217A) designed to slow P_i-release from the active site myosin Va generates a powerstroke a few milliseconds after strongly binding to actin. These findings are most consistent with the powerstroke preceding the release of P_i from the active site. We have also examined how a resistive load affects myosin's rate of P_i-induced detachment from actin. Findings from myosin Va and myosin II indicate that this detachment rate is highly load sensitive, and that a mutation in the switch I region of myosin's active site dramatically alters the load dependence of this rate. Thus, these new finding are providing novel insights into the nature of energy transduction by myosin, which we hope will lead to improved models of muscle contraction and myosin-driven intracellular processes.

SUPER-RESOLUTION SINGLE-MOLECULE IMAGING OF ATP USAGE IN MYOFIBRILS TO STUDY THICK FILAMENT REGULATION

Matvey Pilagov[1]; Sonette Steczina[2]; Ateeqa Niam[1]; Michael Regnier[2]; Michael A Geeves[1]; Neil M Kad[1];
[1]University of Kent, School of Biological Sciences, Canterbury, United Kingdom
[2]University of Washington, Department of Bioengineering, Seattle, WA, USA

Controlling access of myosin to actin in muscle modulates how energy is used. This control occurs at both the thin and thick filaments levels; with the latter involving two states of myosin: disordered relaxed (DRX – able to interact with actin), and super-relaxed (SRX – does not interact with actin). The amount of DRX defines the number of myosins available to interact with actin. However, it is still uncertain how this SRX-DRX equilibrium is distributed within the sarcomere. By fluorescently labelling ATP, we are able to measure the activity of individual myosins within myofibrils. Super-resolution deconvolution provides ~30 nm localization of these activities, enabling assignment to the three zones of the thick filament P, C and D. For fast skeletal muscle (rabbit psoas), we observed 53% of all heads in the C-zone were SRX, compared with 35% and 44% in the P- and D-zones, respectively. This suggests myosin binding protein C (MyBP-C) influences the SRX-DRX equilibrium. To investigate if phosphorylation of MyBP-C affects the population of SRX, we treated myofibrils with protein kinase A (PKA). We found that PKA decreased SRX to 34% in the C-zone, whereas the P- and D-zones were not affected. Nowhere is the control of myosin head accessibility more relevant than in the heart, therefore we have studied the localization of DRX and SRX in porcine cardiac myofibrils, which possess human equivalent beta-cardiac myosin. We will report on the effects of PKA phosphorylation and the recently FDA-approved hypertrophic cardiomyopathy drug, mavacamten, in these myofibrils. In summary, our results directly show that PKA treatment of muscle releases some but not all myosin heads, equally mavacamten increases SRX but does not completely eradicate DRX. Therefore, the activation and repression of myosins within the sarcomere is a combination of multiple competing factors that require an in-depth understanding, especially relevant as we begin to develop drugs to modulate this equilibrium.

MULTI-SCALE MODELING OF MUSCLE BIOPHYSICS: FROM MOLECULES TO POPULATIONS

Andrew D. McCulloch[1,2,4]; Abigail E Teitgen[1]; Marcus Hock[1]; Kimberly J McCabe[5]; Swithin Razu[1]; Annabelle N Fowler[1]; Katherine Knaus[1]; Stephanie Khuu[1]; Matthew C Childers[4]; Bahador Marzban[6]; Gary Huber[3]; J. Andrew McCammon[3]; Daniel A Beard[6]; Michael Regnier[4];
[1]UC San Diego, Bioengineering, La Jolla, CA, USA
[2]UC San Diego, Medicine, La Jolla, CA, USA
[3]UC San Diego, Chemistry and Biochemistry, La Jolla, CA, USA
[4]University of Washington, Bioengineering, Seattle, WA, USA
[5]Simula Resesarch Laboratory, Oslo, Norway
[6]University of Michigan, Molecular and Integrative Physiology, Ann Arbor, MI, USA

Multi-scale modeling of biological systems has advanced rapidly, driven in no small part by exceptional progress in mechanistic modeling of muscle biology and biophysics from molecular to population scales. Advances in deep learning promise to accelerate this progress, so that simulations and analyses previously considered infeasible may soon be routine. Here, we use the biophysics and biomechanics of cardiac and skeletal muscle as exemplars of a multi-scale modeling approach that spans: atomistic resolution models of molecular structure-function relations of myofilament proteins; sub-cellular models of myocyte biophysics; whole cell models of mechano-energetics, metabolism and cell signaling; multicellular agent-based models of cell-cell and cell-matrix interactions regulating tissue state; continuum models of muscle and myocardial biomechanics and biophysics; organ-scale models of muscle-tendon and cardiac biomechanics; system level models of cardiovascular and limb segmental physiology and performance; and image-driven population-based models of heart disease and human movement phenotypes and their relationship to injury and disease progression and outcomes of therapeutic, training and preventative interventions. By considering the technical barriers to bridging these scales and the potential for emerging technologies to address these barriers, a vision for new multi-scale models of muscle and their applications to science and health is proposed. Supported by the Wu-Tsai Human Performance Alliance, NIBIB, NHLBI, and Additional Ventures

CONNEXIN43 RESTORATION ALLEVIATES END-STAGE ALTERATIONS IN ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA/CARDIOMYOPATHY

Farah Sheikh[1]; Jing Zhang[1]; Fabian Zanella[1]; William Bradford[1]; Kyohei Fujita[1]; Matthew Ellis[1]; Ioannis Karakikes[2]; Robert Lyon[1]; Valeria Mezzano[1]; Jason Roberts[3]; Cassiano Carromeu[1]; Yusu Gu[1]; Jody Martin[4]; Alysson Muotri[1]; Melvin Scheinman[3]; Kirk Peterson[1];
[1]University of California San Diego, La Jolla, CA, USA
[2]Stanford University, Palo Alto, CA, USA
[3]University of California San Francisco, San Francisco, CA, USA
[4]University of Illinois, Chicago, Chicago, IL, USA

Reduction of the predominant ventricular gap junction protein, connexin43, is a molecular hallmark that underlies desmosomal mutations/cell-cell junction deficits and arrhythmias associated with arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). However, less is known of the specific role of connexin43 in the end-stage alterations that facilitate myocardial failure and premature death in ARVD/C populations. In this study, we assessed the impact of connexin43 restoration via viral mediated strategies in mouse and human models of ARVD/C harboring end-stage alterations to reveal therapeutic benefits. We show that connexin43 restoration is sufficient to (i) alleviate cardiac dysfunction and prevent arrhythmias as well as prolong lifespan in a severe mouse model of ARVD/C and (ii) rescue cardiomyocyte physiological deficits in ARVD/C human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) that encompassed diverse ARVD/C genetics and disease severity. We show that connexin43 restoration facilitates desmosomal gene and protein expression as well as relocalization to the cell junction in ARVD/C mouse hearts and ARVD/C human cardiomyocytes. In conclusion, we highlight that strategies targeted at increasing full-length connexin43 represents a therapeutic avenue to treat broad and diverse ARVD/C populations.

MODULATORS OF CARDIAC MUSCLE RELAXATION, AND THEIR ROLE IN CARDIAC PATHOLOGY

Jolanda van der Velden;
[1]Amsterdam UMC, Physiology, Amsterdam, The Netherlands

Less than 20 years ago, awareness increased that impaired muscle relaxation underlies different forms of cardiac disease. For a long time, reduced contractility was believed to be the major defect in heart failure, and therapies aimed to enhance force generation of cardiomyocytes. While fibrosis was and still is considered tob e a key player of stiffening of the heart muscle, in recent years it has become evident that several proteins underlie passive tension generation. Studies in cardiac biopsies from patients with heart failure and preserved ejection fraction, previously known as diastolic heart failure, revealed increased passive tension generated by isolated membrane-permeabilized cardiomyocytes. The high passive tension could be normalized to values found control cardiomyocytes by exogenous protein kinase A and protein kinase G. Studies indicate that a deficit in titin phosphorylation underlies increased cardiomyocyte passive tension. In heart failure, complex changes in extracellular remodeling, and titin phosphorylation and isoform composition underlie impaired relaxation of the heart. In addition to myofilament protein changes, cytoskeletal remodeling, and in particular microtubule detyrosination, has been observed in hypertrophic and dilated forms of human cardiac disease. Changes in extracellular, myofilament and cytoskeletal remodeling depend on the initial cause and severity of cardiac disease. During the presentation, an overview will be given of reported changes in human heart disease.

MICROTUBULES ARE VISCOUS REGULATORS OF MYOCARDIAL MOTION

Matthew A. Caporizzo[1];
[1]The University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA

Diastolic dysfunction is a common and untreatable feature of heart failure that arises from increased ventricular stiffness and impaired relaxation. Recent evidence has emerged that the cardiac microtubule network densifies in end-stage heart failure reducing the shortening and relaxation velocities of failing cardiomyocytes. As microtubules are known regulators of cellular mechanotransduction, we hypothesized that microtubule stabilization occurs during the progression of diastolic heart disease (DHD). To determine if microtubule stabilization is involved in the progression of diastolic heart disease, we quantified microtubule network remodeling and its contribution to myocyte active and passive mechanics in rat models of hypertension and diastolic heart failure. Our results indicate that both hypertensive and DHD rats exhibit proportional increases in microtubule network density and detyrosination. Cardiomyocyte size and viscoelastic stiffness was increased in both hypertensive and DHD myocytes while reduced relaxation velocity was only observed in DHD myocytes. Intervention to reduce microtubule detyrosination was sufficient to partially restore myocyte viscoelasticity and contractile velocity. Together these changes indicate that microtubule stabilization occurs in both HTN and DHD and intervention targeting stable microtubules is sufficient to partially reverse myocyte stiffening and impaired relaxation.

REGULATING TITIN BASED STIFFNESS IN HEALTH AND DISEASE

Michael Gotthardt;
[1]Max Delbrück Center for Molecular Medicine, Translational Cardiology and Functional Genomics, Berlin, Germany
[2]Charité Universitätsmedizin Berlin, Department of Cardiology, Angiology and Intensive Care Medicine, Berlin, Germany

The giant Titin protein plays a crucial role in maintaining the passive stiffness of the muscle and the response to mechanical load. Titin based stiffness is tightly regulated by various factors such as post-translational modifications, alternative splicing, and proteolysis. In healthy individuals, this regulation is essential for the normal functioning of the heart and skeletal muscle. However, alterations in titin-based stiffness have been associated with various pathological conditions such as cardiomyopathy, muscular dystrophy, and heart failure. We have used a series of animal models eliminating select spring regions to dissect trophic from mechanosignaling and developed a splice therapeutic approach to reduce titin based stiffness in cardiac disease.

FIBROBLAST-MEDIATED REGULATION OF CARDIAC STRUCTURE & STIFFNESS.

Ross C Bretherton[1]; Isabella M Reichardt[1]; Kristin Zabrecky[2]; Logan Bailey[2]; Darrian Bugg[2]; Timothy McMillen[3]; Wolfgang Linke[5]; Kristina Kooiker[4]; Amy Martinson[2]; Franziska Koser[5]; Michael Regnier[1]; Elizabeth Plaster[2]; Jagadambika Gunaje[2]; Galina Flint[1]; Farid Moussavi-Harami[2,4]; **Jennifer Davis**[1,2];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Laboratory Medicine and Pathology, Seattle, WA, USA
[3]University of Washington, Anesthesiology, Seattle, WA, USA
[4]University of Washington, Medicine/Cardiology, Seattle, WA, USA
[5]University of Münster, Physiology, Münster, Germany

Inherited mutations in sarcomeric genes that decrease cardiomyocyte tension generation are drivers of dilated cardiomyopathy (DCM). Developing precision therapeutics for and mechanistic studies of inherited DCM have been solely focused on cardiomyocytes with negligible attention directed towards fibroblasts despite their role in regulating the best predictor of DCM severity, cardiac fibrosis. Because the ability to reverse or even slow fibrosis remains a major limitation of both standard of care and first in class therapeutics for DCM, this study examined whether cardiac fibroblast-mediated regulation of the heart's mechanical properties is essential for DCM outcomes. Using a mouse model of inherited DCM we found that prior to the onset of fibrosis and dilated myocardial remodeling both the myocardium and extracellular matrix (ECM) stiffen from switches in titin isoform expression, enhanced collagen fiber alignment, and expansion of the cardiac fibroblast population, which we blocked by genetically suppressing p38 specifically in cardiac fibroblasts. This fibroblast-targeted intervention unexpectedly improved the primary myocyte deficit in force generation and reversed ECM and dilated myocardial remodeling. Together these findings challenge the long-standing paradigm that ECM remodeling is a secondary complication to inherited defects in cardiomyocyte contractile function and instead demonstrate cardiac fibroblasts are essential contributors to the DCM phenotype, thus suggesting DCM-specific therapeutics will require fibroblast-specific strategies.

CONTRIBUTION OF THICK FILAMENT STIFFNESS TO LENGTH DEPENDENT ACTIVATION IN CARDIAC MUSCLE

Bradley M Palmer[1]; Anthony L Hessel[2];
[1]University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA
[2]University of Muenster, Institute of Physiology II, Muenster, Germany

Striated muscle features the property of length dependent activation (LDA), which is observed as an increase in contractile force and myofilament calcium sensitivity with increasing sarcomere length (SL). Although the molecular mechanism that underlies LDA is not fully understood, it's well recognized that otherwise unavailable myosin heads are lifted or activated from the thick filament upon its sensing sarcomere stretch via titin. The thick filament also stretches as much as ~1.5% at long SL or with activated force, and thick filament stiffness is concomitantly enhanced 10-100 fold. We asked if this increase in thick filament stiffness due to stretch contributes to LDA by enhancing the transmission efficiency of myosin crossbridge force. To answer this question, we generated a computer model of a half thick filament experiencing forces due to thick filament stretch (F_m), myosin crossbridges (F_{xb}), and the extensible region of titin (F_e). Using published results, the force-stretch characteristics of rat cardiac muscle thick filaments and of rat cardiac titin were compiled, and thick filament stretch was then calculated to satisfy a force balance $F_m = F_{xb} + F_e$ over a range of simulated calcium activation. We found that, if the stiffness of the myosin S2 segment is linear, LDA does not emerge within increasing SL. However, if myosin S2 segment demonstrates increased stiffness in proportion to thick filament stretch, key features of LDA appear. Specifically, maximal developed force is enhanced ~12% as SL is increased from 1.8 to 2.2 mm. Furthermore, apparent calcium sensitivity, pCa_{50}, is enhanced by ~0.02 units, and the apparent Hill coefficient, n, is reduced by ~0.3, as often reported with LDA. These results suggest that the non-linear stiffness characteristics of the thick filament and myosin S2 segment plausibly contribute to LDA in striated muscle.

MULTISCALE MODELS OF MUSCLE MELDING MOLECULAR AND MYOFILAMENT LEVELS OF ORGANIZATION.

Matt Childers[2]; Travis Tune[1]; Farid Moussavi-Harami[3]; Sage Malingen[1,2]; Jennifer Davis[4]; Michael Regnier[2]; **Thomas L Daniel**[1];
[1]University of Washington, Biology, Seattle, WA, USA
[2]University of Washington, Bioengineering, Seattle, WA, USA
[3]University of Washington, Cardiology, Seattle, WA, USA
[4]University of Washington, Pathology, Seattle, WA, USA

Force generation by muscle is brought about by the complex interaction among millions of motor proteins interacting in a highly structured compliant lattice of thick and thin filaments. Muscle contractility is therefore a problem of multi-scale physics: it spans scales from the dynamics that occur at few Angstroms to those that characterize entire cells. Importantly, biophysical processes to that occur at the scale of an entire cell or muscle will influence dynamics that govern rate transitions at the scale of single molecules. Similarly, the single molecule processes at the atomic scale determine the larger scale dynamics. To explore this bi-directional interaction across these spatial scales we combine our spatially explicit model of the half sarcomere with an atomistic scale model of thin filament actin monomers exposed to an externally applied force. The force is computed from the spatially inhomogeneous thin filament forces that arise during activation. The largest forces and strains arise at the M-line and near the Z-disk with lower forces occurring in the middle of the thin filament. The local strains, ranging between 0.1 and 0.2% , is used to estimate the work associated with that strain and guides steered MD models. In structures generated from cryoEM models, helical spacings within an unstretched f-actin heptamer gave rise to actin layer lines measuring helical spacings of 6.0 nm and 5.0 nanometers. This disagreed with helical spacings of 5.9 and 5.1 nm measured in x-ray diffraction of muscle under passive tension. In steered MD simulations, bi-directional axial stretch amounting to 27 kcal/mol work was applied to the heptamers to recover the 5.9/5.1 helical spacings observed experimentally. Deformations of the thin filament from the atomistic scale are then compared to those computed for the sarcomere model.

MULTISCALE MODELING OF CARDIAC GROWTH

Kenneth S. Campbell[1];
[1]University of Kentucky, Gill Heart and Vascular Institute, Lexington, KY, USA

Multiscale models of the cardiovascular system can provide new insights into physiological and pathological processes. MyoVent is a computer model that bridges from molecular to organ-level function and which simulates a left ventricle pumping blood through the systemic circulation. The code is being developed as a testbed for a long-term project that seeks to use computer models to optimize care for patients with cardiac disease. Recently, MyoVent was extended to provide baroreflex control of arterial pressure. This allowed the model to regulate arterial pressure when sarcomere-level function was perturbed via myotropes and/or genetic modifications. This abstract describes a new growth module for MyoVent that allows the ventricle to dilate/constrict (eccentric growth) or thicken/thin (concentric growth) in response to physiological signals. Eccentric growth is mimicked by adding/removing sarcomeres in series around the ventricular circumference. Concentric growth involves the addition/removal of sarcomeres in parallel. Initial calculations have tested different potential growth signals. The best results to date have been obtained by controlling eccentric growth with passive intracellular stress and concentric growth via the intracellular ATP concentration. Simulations based on these feedback laws reproduce ventricular growth patterns induced by clinical conditions including: hypertension, aortic valve stenonsis, mitral valve regurgitation, depressed sarcomere-level contractility, and mechanical unloading via ventricular assist devices. The long-term goal is to extend the modeling framework to develop computer models that clinicians can use to develop personalized therapeutic plans for patients with different forms of cardiovascular disease.

SARCOMERE DYNAMICS SIMULATIONS TO UNCOVER MECHANISMS IN HYPERTROPHIC CARDIOMYOPATHY

Cheavar A. Blair[1]; Alison Vander Roest[2]; Kathleen Ruppel[4]; James Spudich[4]; Regnier Michael[5]; Beth Pruitt[6]; Daniel Bernstein[3];
[1]University of Kentucky, Physiology, Lexington, KY, USA
[2]University of Michigan, Biomedical Engineering, Ann Arbor, MI, USA
[3]Stanford, Pediatrics, Stanford, CA, USA
[4]Stanford, Biochemistry, Stanford, CA, USA
[5]University of Washington, Bioengineering, Seattle, WA, USA
[6]University of California Santa Barbara, Bioengineering, Santa Barbara, CA, USA

Mutations in the beta myosin heavy chain (MYH7) can cause hypercontractility and cardiac remodeling in hypertrophic cardiomyopathy (HCM), but the mechanisms of specific mutations can be difficult to predict. Many mutations change intrinsic rates of myosin activity and their force sensitivity, which can dynamically alter chemo-mechanical cycle events and resultant force generation during cardiac contraction. Utilizing a multiscale approach enables measurement of the kinetics and force generation in purified myosin proteins, isolated myofibrils, and single and multicellular human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) platforms. Computational modeling can integrate results across these scales and provide additional insights into the individual and combinatorial effects of different changes in myosin kinetics. We have previously used a continuum model to highlight the significance of dysregulation of the super relaxed state of myosin driving hypercontractility in hiPSC-CMs with the P710R mutation. Sensitivity analysis also supported variable myofibril density and sarcomeric organization as a source of population heterogeneity in the force-generating capacity of individual hiPSC-CMs. In our ongoing studies, we are leveraging computational modeling to compare the predicted and measured effects of HCM mutations in our different experiments. We have incorporated the measured changes in unloaded myosin to predict force generation after isometric maximal calcium activation (myofibril measurements) and twitch stimulation in single hiPSC-CMs on gels of physiologic stiffness. Additional simulations with spatially explicit models enable more direct prediction of the impact of heterogeneous mixtures of myosins with different molecular profiles and on cooperativity effects associated with the transmission of tension along the length of a sarcomere. Finally, we have visualized the strain profiles of individual sarcomeres in contracting myofibrils in live cells and are correlating these measurements to our simulations. These studies will give new insights into the key biophysical drivers of HCM.

HARNESSING MULTISCALE MODELS TO UNDERSTAND DILATED CARDIOMYOPATHY THIN FILAMENT MUTATIONS

Michael J. Greenberg;
[1]Washington University School of Medicine, Biochemistry and Molecular Biophysics, Saint Louis, MO, USA

transplantation in both adult and pediatric patients. Excellent clinical studies have shown that point mutations in sarcomeric proteins including troponin T are prominent causes of dilated cardiomyopathy; however, genetic information is generally not considered when treating patients, in part, due to challenges connecting genotype and phenotype. Fundamentally, point mutations in proteins affect their structure, function, and/or abundance, but we do not have a good understanding of how molecular dysfunction is related to changes in cellular and tissue function. To begin to address this gap in our knowledge, we have applied multiscale tools to study point mutations in troponin T associated with dilated cardiomyopathy at the molecular, cellular, and tissue levels. We show that while different mutations in troponin T reduce cellular contractility, they do so with different molecular mechanisms. Moreover, we demonstrate how knowledge of molecular mechanism can be harnessed to identify compounds that improve tissue-scale contractility for specific mutations. Taken together, our studies represent an important step in connecting genotype and phenotype across scales.

MACHINE LEARNING FOR BUILDING CLASSIFIERS AND RATE ESTIMATES IN SIMULATED TWITCHES

Travis Tune[1,2]; Anthony Asencio[2,3]; Sage Malingen[2,3]; Kristina Kooiker[1]; Jennifer Davis[3,4]; Thomas Daniel[2]; **Farid Moussavi-Harami**[1,4];
[1]University of Washington, Medicine/Cardiology, Seattle, WA, USA
[2]University of Washington, Biology, Seattle, WA, USA
[3]University of Washington, Bioengineering, Seattle, WA, USA
[4]University of Washington, Laboratory Medicine and Pathology, Seattle, WA, USA

Computational models of the sarcomere allow for understanding mechanisms and modeling disease conditions or drugs. We have used a spatially explicit Monte-Carlo Markov-Chain model to simulate a set of twitches by introducing rate constant perturbations that mimic thin and thick filament activation associated with genetic cardiomyopathies. Harnessing this large, labeled data set, we used machine learning (ML) methods to classify disease states based on a collection of temporal features. These features include the actual time history of simulated twitches, the twitch tension index (TTI, an integral of the twitch), the first two principal components (PCs) based on singular value decomposition and traditional activation/relaxation parameters. Some of these features, notably TTI combined with the raw twitch data and PCs and TTI approach 80% classification accuracy. We extended the model from our earlier analysis of three cross-bridge state transitions to five state transitions along with the super-relaxed state or myosin "off state" – essentially a six-state model modified and then generated even larger data set and varying a larger parameter space. We also implemented improved estimates of state transition probabilities using a newly developed algorithm for computing the matrix exponential of the rate constant matrix. Moreover, the spatially explicit nature of our model also now allows for cooperative activation via nearest neighbor interactions between troponin sites on the thin filament. We have now simulated 500,000 combinations of rate parameters to solve the inverse problem of estimating rate parameters for specified twitches by implementing Bayesian parameter estimation method using a Conditional Variational AutoEncoder to compute the one and two-dimensional marginalized posteriors (probability distributions) that predict the rates. We will apply this technique to identify therapeutic targets for different abnormal twitches.

STRUCTURE OF PREPOWERSTROKE ACTOMYOSIN BY CRYOELECTRONMICROSCOPY AT 10 MS AND 5A

Howard D White[1,2]; Risi Cristina[1]; David Klebl[2]; Stephen Muench[2]; Charlie Scarff[2]; Vitold Galkin[1];
[1]Eastern Virginia Medical Center, Physiological Sciences, Norfolk, VA, USA
[2]Leeds University, Astbury Center, Leeds, United Kingdom

We have determined the structure of the actomyosin complex formed 10 ms and 120 ms after mixing myosin-ADP-Pi with f-actin. High-resolution studies of actomyosin have been limited to equilibrium conditions with either ADP bound or an empty active site and the structure of the short lived primed, prepowerstroke, structure of the catalytic cycle has previously remained elusive. Here, using millisecond time-resolved cryo-electron microscopy we have determined the structure of the prepowerstroke actomyosin complex formed 10 ms after mixing myosin-ADP-Pi with f-actin. The predominant structure at 10 ms reveals myosin binding the actin filament through its lower 50 kDa subdomain with only the loop2 interacting with the actin surface. The predominant structure 120 ms after mixing with actin is a second conformational state in which the actin-binding cleft is closed and the lever is in a post-power stroke position, similar to previous strongly-bound actomyosin structures. Together, the two states represent the start and end positions of the powerstroke and allow us to assemble the most complete picture of the actomyosin catalytic cycle to date.

MULTI-SCALE MODELING ACROSS MUSCLES, CONTEXTS, AND DISEASES

Silvia Blemker
University of Virginia, USA

No Abstract

MULTISCALE MUSCLE MODELING: AN ORGANISMAL APPROACH

Kiisa Nishikawa[1]; Monica A Daley[2]; Jill McNitt-Gray[3]; Anne Silverman[4]; Simon Sponberg[5]; Madhusudhan Venkadesan[6];
[1]Northern Arizona University, Biological Sciences, Flagstaff, AZ, USA
[2]University of California at Irvine, Ecology & Evolutionary Biology, Irvine, CA, USA
[3]University of Southern California, Health Sciences, Los Angeles, CA, USA
[4]Colorado School of Mines, Mechanical Engineering, Golden, CO, USA
[5]Georgia Institute of Technology, Physics, Atlanta, GA, USA
[6]Yale University, Mechanical Engineering, New Haven, CT, USA

Bottom-up approaches to muscle modeling have emphasized properties including calcium and cross-bridge kinetics based on parameters measured from in vitro and ex vivo experiments. These models perform fairly well at predicting forces produced by individual muscles during steady-state in vivo movements, but they perform much less well under dynamical and unsteady perturbed conditions. Recent in vivo measurements of unsteady, perturbed locomotion demonstrate 1) weak coupling of both timing and magnitude of muscle force from activation dynamics, and 2) the importance of strain dynamics (i.e., abrupt changes in strain rate) and interactions between strain dynamics and activation on the timing and magnitude of muscle force production. These effects are not explained by the current paradigm nor are they accurately predicted by current models, in part because experiments used to validate and refine those models have focused on isometric, isotonic and steady-state conditions. By performing ex vivo experiments that emulate changes in loading and strain rates that emulate in vivo unsteady locomotion, we demonstrate that isometric, isotonic and sinusoidal oscillations fail to predict in vivo muscle mechanics. We further show that strain transients and interactions between strain transients and activation have large effects on muscle force and work. These interactions suggest the view that muscle is a tunable active material where its force response to deformation depends on activation. The data help to reconcile CNS vs. preflex control of muscle force by suggesting that strain dynamics may provide tuning points at which muscles are most sensitive to activation. To advance understanding of muscle mechanics and improve the accuracy of multiscale models, we need to design multiscale experiments that describe interactions between dynamically varying strain and activation and extend existing models and theories to account for their interactions.

EMERGENT RHEOLOGY OF ACTOMYOSIN ENSEMBLES

Madhusudhan Venkadesan[1]; Dan Rivera[1]; Khoi Nguyen[1]; Boris Shraiman[2];
[1]Yale University, Department of Mechanical Engineering and Materials Science, New Haven, CT, USA
[2]University of California, Santa Barbara, Department of Physics, Kavli Institute for Theoretical Physics, Santa Barbara, CA, USA

Muscle can transition between fluid-like and solid-like mechanical behavior depending upon its excitation and the external load. The isotonic force-velocity curve exemplifies the fluid-like behavior; load-dependence of the strain-rate is reminiscent of fluid rheology. However, for close to isometric force, muscle is solid-like and has negligible strain-rates for timescales 100–1000 times the intrinsic time constants associated with actomyosin dynamics. These rheological transitions are crucial for animal motor control capabilities and emerge from the molecular machinery that constitute muscle. We analyzed actomyosin ensemble models, ranging from two-state myosin models to more complex five-state models, to pinpoint the source of these transitions and found that rheological transitions are an impossibility in current mechanochemical models of actomyosin ensembles. Furthermore, Edman [1] showed that muscle's isotonic response shows nearly zero velocity for a range of loads around 0.8–1.3 times the isometric force, a property that leads to a solid-like rheology and behavior that is reminiscent of stiction (static friction). We found that this stiction-like state cannot be realized by current muscle models, whether they use Huxley's simplified description of the actomyosin cycle or use spatially explicit computer simulations that include titin and myosin-binding protein C (MyBP-C). Our findings show an open challenge in understanding the stiction-like behavior of muscle. Based on further analyses of spatially explicit models we propose a new hypothesis that elastic strain inhomogeneities within a sarcomere, partly influenced by titin and MyBP-C, may underlie emergent mechanochemical dynamics of actomyosin ensembles that lead to excitation-dependent rheological transitions. Acknowledgements NSF grants PHY-1748958, EFMA-1830870, and the Gordon and Betty Moore Foundation Grant 2919.02.References[1] K. A. Edman. The Journal of Physiology 404, 301 (1988).

MODELING MUSCLE COORDINATION IN WHOLE BODY MOVEMENT

Anne Silverman[1,2];
[1]Colorado School of Mines, Mechanical Engineering, Golden, CO, USA
[2]Colorado School of Mines, Quantitative Biosciences and Engineering, Golden, CO, USA

Muscle action drives movement dynamics. The functional roles of muscles are dependent on movement task, and organisms have a remarkable ability to achieve a wide variety of movements by adapting coordination of multiple muscles. Movement tasks have multiple and changing objectives including maintaining stability, supporting body weight, and accelerating body segments. Muscle mechanics are challenging to characterize, however, as their forces and velocities are difficult to directly measure. Musculoskeletal modeling and simulation techniques incorporate multiple muscles that coordinate to drive observed movement. We use optimization approaches to determine the muscle recruitment solution for the redundant musculoskeletal system. These approaches allow investigation of impaired and at-risk populations to evaluate device design and treatment interventions. We have identified muscle compensations that help achieve challenging task dynamics in the face of musculoskeletal deficits, but may also contribute to long-term risk of injury in muscles and joints. For example, runners with a unilateral transtibial amputation compensate for lost energy generation from the ankle plantar flexors using their hip extensors to generate positive muscle work to run faster. This greater muscle force generation contributes to higher hip joint loading compared to runners without an amputation, which may result in greater levels of joint degeneration and pain over long durations. In military service members, the demand for hip extensor muscle forces is especially high when carrying heavy backpacks up steep walking slopes. Due to their differing architecture, hamstring muscles produce different levels of concentric and eccentric muscle work, explaining disparate injury prevalence in these muscles. To maximize human performance and minimize injury risk, we must characterize multi-muscle coordination effectively at the organism level. Furthermore, incorporating variation across individuals will improve personalized training and treatment.

AN EXPERIMENTAL APPROACH TO MANIPULATE THE MULTI-SCALE COMPONENTS OF WHOLE-BODY MOVEMENT THAT INFLUENCE SINGLE MUSCLE FIBER WORK AND POWER OUTPUT

David C Lin[1,2]; **Bertrand C.W. Tanner**[1,2];
[1]Washington State University, Integrative Physiology and Neuroscience, Pullman, WA, USA
[2]Washington State University, Voiland School of Chemical Engineering and Bioengineering, Pullman, WA, USA

Locomotion includes coupling between muscles, tendons, bones, and joints, which intrinsically form a multi-scale mechanical feedback system. Namely, the forces and length changes that occur in a muscle fiber are influenced by muscle architecture, mechanical characteristics of the attached tendon, musculoskeletal geometry, and inertias of the limbs and body. To probe how muscle performance is affected by these elements, it is necessary to manipulate the properties of these elements to better understand system coupling. While this manipulation is possible in computational models, experimentally manipulating these elements is difficult, especially as the scale becomes larger and the system contains more elements. To address this challenge, we have developed a virtual mechanical load environment for single skeletal muscle fibers based on the concept of mechanical impedance and implemented by force-feedback. Within this environment, we can manipulate crossbridge mechanisms via solution-based methods, as well as muscle architecture, tendon, and inertial properties. These manipulations can be either parametric (e.g., increasing tendon compliance) or introduce nonlinear behavior (e.g., including the “toe” region of tendon). Our initial study addressed how tendon compliance influences muscle power and work output as a fiber is activated from rest (relaxed conditions). We tested compliance values spanning a 50-fold change, while activating chemically-skinned fibers from a rat medial gastrocnemius. Power output from the fiber increased nonlinearly with increases in tendon compliance. Interestingly, there was not a large change in muscle force throughout the movement for the different compliances, implying force-velocity effects were not prominent, potentially due to the activation-driven force increase from rest. Future studies will examine how the toe region of tendon and rotation of fibers within a whole muscle (i.e., changes in pennation angle) influence muscle power generation.

POSTER ABSTRACTS

Monday, July 17
POSTER SESSION I
16:30 - 18:00
Sibson Foyer

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Monday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 16:30 – 17:15 | Even-Numbered Boards 17:15 – 18:00

Chakraborti, Ananya	**1 – POS**	**Board 1**
Chung, Charles	**4 – POS**	**Board 4**
Gebauer, Amadeus	**7 – POS**	**Board 7**
Kekenes-Huskey, Peter	**10 – POS**	**Board 10**
Lewalle, Alexandre	**13 – POS**	**Board 13**
Marcucci, Lorenzo	**16 – POS**	**Board 16**
Nesmelov, Yuri	**19 – POS**	**Board 19**
Palmer, Bradley	**22 – POS**	**Board 22**
Prodanovic, Momcilo	**25 – POS**	**Board 25**
Seetharamaiah, Attili	**28 – POS**	**Board 28**
Teitgen, Abigail	**31 – POS**	**Board 31**

Posters should be set up on the morning of Monday, July 17 and removed by noon on Thursday, July 20. All uncollected posters will be discarded.

1-POS Board 1

COMPUTATIONAL INSIGHTS INTO THE EFFECT OF MAVACAMTEN ON THE ATP HYDROLYSIS STEP OF THE HUMAN CARDIAC BETA MYOSIN

Ananya Chakraborti[1]; Jil C Tardiff[2]; Steven D Schwartz[1];
[1]University of Arizona, Department of Chemistry and Biochemistry, Tucson, AZ, USA
[2]University of Arizona, Department of Biomedical Engineering, Tucson, AZ, USA

Mavacamten is a cardiac specific allosteric modulator observed to inhibit myosin ATPase activity. It directly binds to cardiac beta myosin altering multiple steps of the cross-bridge cycle. In this detailed study, we aimed to explore the atomistic alterations in the ATP hydrolysis step of the human cardiac beta myosin due to the presence of Mavacamten using the rare-event method Transition Path Sampling. The crystal structures of the Mavacamten-bound myosin are not yet available and hence, we have utilized the molecular docking approach to develop the initial probable structure of the same in the pre-powerstroke conformation. We then generated unbiased thermodynamic ensemble of reactive trajectories for the breakdown of ATP to Adenosine diphosphate (ADP) and Hydrogen Phosphate (HPO_4^{2-}). Our results were able to predict the variations due to Mavacamten, in the active site as well as the general pathway of the hydrolysis reaction and the transition states involved. We have also included the mutant forms of myosin causing genetic cardiomyopathies: R712L and P710R to detect the effect of the drug on the mutation-induced changes in the chemical step. These molecular insights are essential to understand the drug action and hence, aid in their targeted applications.

4-POS Board 4

RAMP-STRETCHES DURING RELAXATION OF TWITCHING INTACT CARDIAC TRABECULA SUGGEST A NEED FOR DYNAMIC MODELS OF MYOFILAMENT FUNCTION

Bertrand C Tanner[1]; Bradley M Palmer[2]; **Charles S Chung**[3];
[1]Washington State University, Integrative Physiology and Neuroscience, Pullman, WA, USA
[2]University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA
[3]Wayne State University, Physiology, Detroit, MI, USA

We have previously found that relaxation is dependent on the strain rate of a lengthening stretch just prior to relaxation, also referred to as Mechanical Control of Relaxation. To investigate the mechanisms underlying Mechanical Control of Relaxation, we sought to characterize experimentally induced ramp-stretches using existing models of stress-responses to muscle stretch. Ramp-stretches of varying strain rates (amplitude=1% muscle length) were applied to intact rat cardiac trabeculae following a load-clamp at 50% of the maximal developed twitch force, which provide a first-order estimate of ejection and coupling to an afterload. The resultant stress-response was calculated as the difference between the time-dependent stress profile between load-clamped twitches with and without a ramp-stretch. The stress-response exhibited features of the step-stretch response of activated, permeabilized myocardium, such as distortion-dependent peak stress, rapid force decay related to crossbridge detachment, and stress recovery related to crossbridge recruitment. The peak stress was strain rate dependent, but the minimum stress and the time-to-minimum stress values were not. As the stretches occurred later into diastole, a more passive stress-response was observed. Three mathematical models with parameters representing crossbridge attachment and detachment kinetics were fit to the stretch responses to assess whether crossbridge kinetics showed a strain rate-dependence, as predicted by prior studies (Kawai and Brandt 1980, Palmer et al 2007, Palmer et al 2020). An explicit strain-dependence in the kinematic model suggests that crossbridge detachment rates increase as strain rates increase, but all models showed some limitation the fits, especially near the nadir of the stress response. A substantive limitation of these models is likely the assumption of constant calcium and thin filament activation. Additionally, models including more explicit (fewer lumped) parameters and/or time-varying changes (Smith and Geeves 1995, Campbell 2014) may provide improved mechanistic insight into strain-rate dependent changes of myofilaments during relaxation.

7-POS Board 7

A CONSTRAINED MIXTURE MODEL OF SARCOMERE TURNOVER IN CARDIOMYOCYTES FOR ORGAN-SCALE CARDIAC GROWTH AND REMODELING

Amadeus M Gebauer[1]; Martin R Pfaller[2,3,4]; Wolfgang A Wall[1];
[1]Technical University of Munich, Institute of Computational Mechanics, Munich, Germany
[2]Stanford University, Pediatric Cardiology, Stanford, CA, USA
[3]Stanford University, Stanford Maternal & Child Health Research Institute, Stanford, CA, USA
[4]Stanford University, Institute for Computational and Mathematical Engineering, Stanford, CA, USA

Changes in the mechanical environment in the heart wall (e.g., caused by cardiovascular diseases) trigger cardiac growth and remodeling (G&R), eventually leading to heart failure in many patients. An important mechanism of G&R in soft tissue is turnover, which is the continual deposition and degradation of tissue constituents.We propose a novel model of sarcomere turnover in cardiomyocytes based on the constrained mixture model and a rheological model of sarcomeres. The rheological model of the sarcomeres includes passive and active contributions. Sarcomeres continuously replace themselves or even grow or shrink in number when cardiomyocytes are perturbed from their preferred mechanical environment. Existing models often do not explicitly model the effect of turnover on the active part of the tissue. We combine our novel sarcomere turnover model with the existing G&R model of the extracellular matrix in our finite-element-based simulation framework.We apply our model to a patient-specific bi-ventricular heart and show how changes in the mechanical environment of cardiomyocytes induced by different overload conditions result in organ-scale G&R. We identify mechanobiological stable and unstable growth depending on the severity of hypertension and different growth factors. Furthermore, we elaborate on how our model predicts the reversal of G&R after returning blood pressure to baseline.Our microstructure-motivated model of organ-scale cardiac G&R, together with experimental data from biomimetic cultures of living human myocardium and clinical data of long-term cardiac magnetic resonance imaging of patients has the potential to not only increase understanding but also identify patients at risk of heart failure and assess or even improve their personalized therapy options.

10-POS Board 10

MOLECULAR MECHANISMS OF CARDIAC REGULATION THROUGH MODIFYING INTRINSICALLY DISORDERED REGIONS IN MYOFILAMENT PROTEINS

Peter Kekenes-Huskey;
[1]Loyola University Chicago, Maywoo, IL, USA

The heart adapts to cardiac demand through a variety of mechanisms. Some of these adaptions include chemical modifications of myofilament proteins responsible for cell contraction. Interestingly, many of these chemical modifications, such as phosphorylation and acetylation, are found in unstructured, or intrinsically-disordered, regions of proteins. However, it has been surprisingly difficult to determine how modifications of intrinsically disordered regions (IDRs) influence the function of their parent proteins or the myofilament as a whole. We hypothesized that regulation of myofilaments proteins via their IDRs occurs because post-translational, e.g. chemical, modifications (PTMs) alter their conformation ensembles. To evaluate this hypothesis, we used molecular dynamics and continuum models for simulating a representative IDR to describe its conformation ensemble before and after modification. Our results indicate that local changes in the physicochemical properties of an IDR via chemical modification can influence global ensemble properties. These findings provide an important clue into the mechanisms of myofilament protein regulation through PTMs.

13-POS Board 13

MODELLING THE FUNCTIONAL IMPLICATIONS OF THICK-FILAMENT OFF-STATE DYNAMICS IN CARDIAC MUSCLE

Alexandre Lewalle[1]; Gregory N Milburn[2]; Kenneth S Campbell[2]; Steven A Niederer[1];
[1]King's College London, Biomedical Engineering, London, United Kingdom
[2]University of Kentucky, Department of Physiology, Lexington, KY, USA

The regulation of cardiac sarcomere contraction is essential for ensuring physiological function. Conventional descriptions of tension regulation have focused predominantly on thin-filament properties, but growing experimental evidence indicates a complementary thick-filament-based activation. This process involves a structural transformation from a super-relaxed "off" state where myosin heads cannot interact with the actin thin filaments. This transition is itself tension-dependent, implying a positive feedback mechanism. The extent to which this mechanism accounts for length-dependent activation (LDA) and the Frank-Starling effect, alongside other sarcomere-level mechanisms, remains to be fully established. This challenge stems, in part, from the non-trivial coupling between strain and stress within the sarcomere, making the distinction between explicit and implicit length dependence ambiguous. Long before the discovery of the off state, some mathematical models of myocyte contraction were implementing length dependence by manually inserting phenomenological strain-dependent terms in, e.g., tension magnitude, calcium sensitivities, or rate constants. Whilst enabling adequate empirical fits to measurements, this ad hoc approach does not necessarily embody specific biophysical mechanisms systematically. The aim of this study was therefore to test whether, in principle, off-state dynamics could plausibly account, either fully or partially, for LDA without resorting to ad hoc phenomenological formulations. We amended the Land 2017 ODE contraction model of the human left ventricle to include force-dependent off-state dynamics, and tested its ability to reproduce effective length-dependent steady-state (F-pCa) and dynamic (ktr) behaviour. Our results qualitatively reproduced essential observable features of length-dependent calcium sensitivity, force magnitude, and ktr. We tested and discarded alternative hypothetical feedback scenarios based explicitly on strain. We modelled the off-state-stabilising drug mavacamten, reproducing observed length-dependent steady-state and ktr behaviours. In conclusion, our results support the plausibility of myosin off-state dynamics as a potentially dominant contributor to sarcomere tension regulation and LDA.

16-POS Board 16

A MATHEMATICAL MODEL OF THE DUAL-FILAMENT REGULATION FEEDBACK SYSTEM IN SKELETAL MUSCLE

Lorenzo Marcucci[1,2]; Luca Fusi[3,4];
[1]University of Padova, 1Department of Biomedical Sciences, Padova, Italy
[2]RIKEN, Center for Biosystems Dynamics Research, Suita, Japan
[3]King's College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom
[4]King's College London, Centre for Human and Applied Physiology, London, United Kingdom

Beside the classic calcium-mediated regulation of muscle contraction, driven by structural changes in the thin filaments regulatory proteins, a second pathway is emerging, based on the activation of the thick filaments, whose myosin motors in the relaxed state are in an auto-inhibited configuration, lying along its surface. The proposed mechanosening mechanism postulates that the level of activation of the thick filament is regulated by the level of tension which it sustains. Therefore, few constitutively active motors can sense the activation of the thin filaments and generate force through the actomyosin cyclical interaction, which in turns activates the remaining motors in a cooperative way. Moreover, attached myosin motors can further stabilize the active conformation of the thin filament regulatory proteins, forming a two-way feedback system. This two-way feedback system increases the complexity of the fine regulation of muscle contraction, making it adaptable to a variety of tasks, but also obscuring its role in the physiological contraction. In this respect, mathematical modeling is useful to dissect the role of the different components of the muscle regulation. Here, we combine mechanical and structural data during activation in near-physiological conditions, using calcium jumps produced by photolysis of caged calcium and probes on myosin in the thick filaments to monitor its activation states, with a mathematical model which includes the mechanosening mechanism tightly constrained on the activation rates previously observed under passive forces on the same muscle and conditions. Quantitative simulations suggest that the initial phases of contraction are driven by about a 35% of constitutively active motors, a value coherent with previous estimations. However, at filament stresses higher than 0.5 T0, the model without a myosin-induced activation of the thin filament predicts a slower activation of the thick filament with a reduced fitting of the rate of force development. A discrepancy can also be seen in the experimental data comparing the rate of force development and re-development after a fast, small, imposed shortening at T0. We show that including the two-way feedback is necessary to properly describe muscle force generation during a tetanic contraction.

19-POS Board 19

COOPERATIVE BINDING OF MYOSIN-NUCLEOTIDE COMPLEX AND UNREGULATED F-ACTIN

Aarushi Naskar[1]; Alexis Johnson[1]; Mitchell Turk[1]; **Yuri Nesmelov**[1];
[1]UNC Charlotte, Physics, Charlotte, NC, USA

In the actomyosin cycle myosin hydrolyses ATP and binds actin, initially weakly and then strongly. The timing of myosin ATP hydrolysis and subsequent binding to actin remains elusive. We performed a double-mixing transient experiment to characterize the kinetics of actomyosin interaction shortly after ATP binding to myosin. In the experiment we first rapidly pre-mix rabbit skeletal myosin S1 and equimolar ATP, thus producing myosin-ATP complex. After a controlled delay we rapidly add unregulated F-actin to the mixture to observe the interaction of actin with myosin-ATP complex. We detect transients of the light scattering after the second mixing. A sigmoid-shaped curve of transients was observed, which is a sign of cooperative binding. This is in contrast to an exponential curve, usually observed when myosin S1 is rapidly mixed with unregulated F-actin without the presence of any nucleotide, as expected for the well-known strong non-cooperative binding of actin and myosin. We consider several models to explain the observed transients of actin and myosin-ATP complex: a non-cooperative binding, the cooperative single-contiguous and doubly-contiguous actomyosin binding. We conclude that the model of the doubly-contiguous actomyosin binding fits best the observed transients. We conclude that in the presence of physiological ATP concentration, myosin binds actin cooperatively, even without the regulatory proteins of the thin filament.

22-POS Board 22

CONTRIBUTION OF THICK FILAMENT STIFFNESS TO LENGTH DEPENDENT ACTIVATION IN CARDIAC MUSCLE

Bradley M Palmer[1]; Anthony L Hessel[2];
[1]University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA
[2]University of Muenster, Institute of Physiology II, Muenster, Germany

Striated muscle features the property of length dependent activation (LDA), which is observed as an increase in contractile force and myofilament calcium sensitivity with increasing sarcomere length (SL). Although the molecular mechanism that underlies LDA is not fully understood, it's well recognized that otherwise unavailable myosin heads are lifted or activated from the thick filament upon its sensing sarcomere stretch via titin. The thick filament also stretches as much as ~1.5% at long SL or with activated force, and thick filament stiffness is concomitantly enhanced 10-100 fold. We asked if this increase in thick filament stiffness due to stretch contributes to LDA by enhancing the transmission efficiency of myosin crossbridge force. To answer this question, we generated a computer model of a half thick filament experiencing forces due to thick filament stretch (F_m), myosin crossbridges (F_{xb}), and the extensible region of titin (F_e). Using published results, the force-stretch characteristics of rat cardiac muscle thick filaments and of rat cardiac titin were compiled, and thick filament stretch was then calculated to satisfy a force balance $F_m = F_{xb} + F_e$ over a range of simulated calcium activation. We found that, if the stiffness of the myosin S2 segment is linear, LDA does not emerge within increasing SL. However, if myosin S2 segment demonstrates increased stiffness in proportion to thick filament stretch, key features of LDA appear. Specifically, maximal developed force is enhanced ~12% as SL is increased from 1.8 to 2.2 mm. Furthermore, apparent calcium sensitivity, pCa_{50}, is enhanced by ~0.02 units, and the apparent Hill coefficient, n, is reduced by ~0.3, as often reported with LDA. These results suggest that the non-linear stiffness characteristics of the thick filament and myosin S2 segment plausibly contribute to LDA in striated muscle.

25-POS Board 25

DO TEMPERATURE AND STIMULATION FREQUENCY MATTER WHEN RECORDING CARDIAC TWITCHES?

Momcilo Prodanovic[1,2,3]; Andjela Grujic[3,4]; Corrado Poggesi[5]; Michael Regnier[6]; Thomas C Irving[7]; Michael A Geeves[8]; Srboljub M Mijailovich[3];
[1]University of Kragujevac, Faculty of Engineering, Kragujevac, Serbia
[2]Institute for Information Technologies, University of Kragujevac, Department of technical and technological sciences, Kragujevac, Serbia
[3]FilamenTech, Inc., Newton, MA, USA
[4]University of Belgrade, Faculty of Physics, Belgrade, Serbia
[5]University of Florence, Department of Experimental & Clinical Medicine, Florence, Italy
[6]University of Washington, Department of Bioengineering, Seattle, WA, USA
[7]Illinois Institute of Technology, Department of Biology, Chicago, IL, USA
[8]University of Kent, Department of Biosciences, Canterbury, United Kingdom

Functional changes of cardiac muscle, triggered by mutations in sarcomere proteins, are typically assessed in experiments with trabeculae from transgenic rodent models while experiments on human cardiac tissue are scarce. Mechanical responses to intracellular calcium in trabeculae are usually assessed as transient twitch contractions. Studies of twitch contractions in either rodent or human cardiac muscle, however, are often done at temperatures and frequencies that significantly differ from physiological which can make interpretation problematic. Extrapolating results from rodents to humans can also be difficult due to the differences in physiological ranges of frequencies and myosin α and β isoform content. We are developing a novel methodology to translate findings from rodents to humans using the MUSICO computational simulation platform. The MUSICO platform embodies detailed schemes for crossbridge cycling, thin and thick filament regulation by calcium, an explicit sarcomere 3D geometry allowing for spatially randomly distributed mixture of myosin isoforms and structural features specifically adjusted for each species. As such, it is well suited as a tool to translate findings between species. Using these simulations, tightly coupled with the experiments, we quantitatively estimated the effects of temperature and stimulation frequency on tension transients in rat trabeculae. We compared model predictions with a consistent set of data from twitches in rat trabeculae which contained simultaneously recorded calcium transients and tension responses. The crossbridge cycle parameters were adjusted for temperature and used for simulations at different frequencies. The predicted twitch responses, matched with the experimental observations, served as a matrix for translation of parameters and calcium transients between species, as for example, simulating functional changes in human ventricular trabeculae when the muscle is stimulated at different frequencies and temperatures. This methodology can be extended to predict functional changes in human cardiac muscle with disease.

28-POS Board 28

HIGH THROUGHPUT ANALYSIS OF THE SUPER-RELAXED STATE OF MYOSIN IN HUMAN MYOCARDIUM

Seetharamaiah Attili; Thomas Kampourakis[1]; Kenneth S Campbell[2];
[1]Kings College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom
[2]University of Kentucky, Department of Physiology and Division of Cardiovascular Medicine, Lexington, KY, USA

Heart muscle contractility is controlled by regulatory structural changes in the myosin-containing thick filaments in addition to the classical thin filament-based mechanisms of regulation, whereby 'myosin motors' or 'myosin head domains' transit between OFF (low ATPase activity and unable to bind to actin) and ON states (higher ATPase activity and high probability to bind actin). When a muscle fibre is not activated, myosin motors are 'parked' in a super-relaxed (SRX) or OFF state, a low ATP consuming configuration. The SRX was found to account for a significant proportion of myosin heads in cardiac thick filaments and its dysregulation is pathogenic in heart diseases such as Hypertrophic Cardiomyopathy (HCM). Modulation of the myosin SRX state has become a central theme for the development of targeted therapeutics for cardiomyopathies and heart failure. For instance, Mavacamten, an allosteric modulator that inhibits ATP turnover is known to be a first FDA-approved drug for the treatment of obstructive HCM. However, the development of novel small molecule effectors that directly target the SRX state of cardiac myosin is hampered by the lack of in-vitro screening assays that accurately reproduce its function. In the current study we have developed a high throughput screening method for the SRX state of cardiac myosin, which is based on mant-ATP pulse-chase methodology. Using this assay, we will test the impact of different small molecule effectors on myosin head confirmation in isolated human myocardium from both human donors, and ischemic and non-ischemic heart failure patients.

MULTISCALE MODELING OF THE EFFECTS OF 2-DEOXY-ATP ON SERCA FUNCTION

Abigail E Teitgen[1]; Marcus Hock[1]; Kimberly J McCabe[2]; Gary Huber[3]; Rommie E Amaro[3]; J. Andrew McCammon[3]; Michael Regnier[4]; Andrew D McCulloch[1];
[1]University of California, San Diego, Bioengineering, San Diego, CA, USA
[2]Simula Research Laboratory, Computational Physiology, Oslo, Norway
[3]University of California, San Diego, Chemistry and Biochemistry, San Diego, CA, USA
[4]University of Washington, Bioengineering, Seattle, WA, USA

2-deoxy-ATP, a naturally occurring near analog of ATP, is a myosin activator that has been demonstrated to increase contractile force and improve ventricular function, including increased relaxation. Calcium transients of cardiomyocytes with elevated levels of dATP show faster decay, but the mechanisms behind this are unknown. We hypothesize that dATP acts on the sarcoendoplasmic reticulum calcium transport ATPase (SERCA2a) pump to accelerate calcium sequestration into the sarcoplasmic reticulum during cardiac relaxation. In this work, we utilized a multiscale computational modeling approach to assess the effects of dATP on SERCA at the molecular and cellular levels. We conducted 200 ns Gaussian accelerated Molecular Dynamics simulations of human cardiac SERCA2a in the apo E1 state, and with ATP and dATP bound. Conformational clustering led to distinct representative structures for ATP- and dATP-bound SERCA. Specifically, dATP showed more stable contacts in the nucleotide binding pocket, and was positioned to allow for faster phosphorylation of the pump than ATP. dATP binding also led to increased closure of the A and N cytosolic domains, suggesting that dATP enhances SERCA pump function via the E1-ATP to E1-ADP transition. dATP also stabilized key transmembrane helices, with greater distances observed between residues in the calcium entry pathway and binding site I. Brownian dynamics (BD) simulations were utilized to assess differences in nucleotide binding kinetics between ATP and dATP to SERCA. Additional BD simulations were used to investigate differences in the rate of calcium binding to ATP- and dATP-bound SERCA. We integrated these results in a model of cardiomyocyte calcium dynamics and found that the molecular level effects of dATP on SERCA contributed to the increased calcium transient decay rate observed experimentally. This study provides the first biophysical evidence of mechanistic differences in SERCA pump function caused by dATP.

Tuesday, July 18
POSTER SESSION II
13:15 - 14:45
Sibson Foyer

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Tuesday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 13:15 – 14:00 | Even-Numbered Boards 14:00 – 14:45

Chakraborti, Ananya	**2 – POS**	**Board 2**
Colson, Brett	**5 – POS**	**Board 5**
McAfee, Quentin	**8 – POS**	**Board 8**
Lehman, Sarah	**11 – POS**	**Board 11**
Malingen, Sage	**14 – POS**	**Board 14**
Mohran, Saffie	**17 – POS**	**Board 17**
Noureddine, Maya	**20 – POS**	**Board 20**
Pathak, Divya	**23 – POS**	**Board 23**
Pruitt, Beth	**26 – POS**	**Board 26**
Tanner, Bertrand	**29 – POS**	**Board 29**
Timir, Weston	**32 – POS**	**Board 32**

Posters should be set up on the morning of Monday, July 17 and removed by noon on Thursday, July 20. All uncollected posters will be discarded.

2-POS Board 2

USING NEURAL NETWORKS TO PREDICT PATHOGENICITY OF VARIANTS IN THE CARDIAC THIN FILAMENT

Ananya Chakraborti[1]; Allison B Mason[1]; Jil C Tardiff[2]; Steven D Schwartz[1];
[1]University of Arizona, Department of Chemistry and Biochemistry, Tucson, AZ, USA
[2]University of Arizona, Department of Biomedical Engineering, Tucson, AZ, USA

Single point mutations to the different proteins of the cardiac thin filament complex can be associated with loss of functionality, generally resulting in genetic cardiomyopathies. Currently, linking genotype to phenotype to determine the pathogenicity of the variants is difficult. Previously, our group used the molecular dynamics simulations to determine the baseline of pathogenic variations found via analysis of computational observables. The pathogenic criterion was then used to predict the pathogenicity of variants of unknown significance (VUS). In this study, we aimed to create a convolutional neural network (CNN) model to predict the pathogenicity of VUS using the results of the molecular dynamics simulations of the full computational model of cardiac thin filament. The basic algorithm consists of several 2D convolution and max pooling layers to reduce the dimensionality of the molecular dynamics data, followed by a dense network and rectified linear unit (ReLU) activation function, finally giving the output. Our neural network model will be able to predict whether a new set of VUS on cardiac troponin T, cardiac troponin I and tropomyosin are benign or pathogenic, with the pathogenic predictions further split into either hypertrophic or dilated cardiomyopathies. These predictions will aid the clinicians to prescribe the appropriate clinical treatment.

5-POS Board 5

HYPERTROPHIC CARDIOMYOPATHY MUTATIONS IN CARDIAC MYOSIN-BINDING PROTEIN C N-TERMINAL DOMAINS CAUSE LOCAL AND ALLOSTERIC EFFECTS ON PROTEIN MOBILITY AND INCREASE ACTIN BINDING

Rhye-Samuel Kanassatega[1]; Fiona L Wong[1]; Thomas A Bunch[1]; L. Michel Espinoza-Fonseca[2]; **Brett A Colson**[1];
[1]University of Arizona, Cellular & Molecular Medicine, Tucson, AZ, USA
[2]University of Michigan, Internal Medicine, Ann Arbor, MI, USA

Mutations in the gene encoding cardiac myosin-binding protein C (cMyBP-C) are a leading cause of hypertrophic cardiomyopathy (HCM). HCM affects more than 1 in 500 individuals and is a leading cause of death in young individuals. However, the mechanisms leading to cardiac dysfunction in HCM remain unclear and therapies are limited. We have used molecular dynamics (MD) simulations and biochemical and biophysical approaches to gain insight into the molecular mechanisms by which mutations in cMyBP-C cause HCM disease. cMyBP-C N-terminal domains C0 through C2 (C0-C2) are considered to be the "business end" of the molecule as they contain regions for binding actin and myosin and phosphorylation, which are important for cMyBP-C's critical roles in normal cardiac function. We selected three HCM-causing mutations in C0-C2 expected to be pathogenic according to the SHaRe Registry and NIH ClinVar databases to investigate: P161S and Y237S in the hydrophobic core of domain C1 and surface-exposed P371R in domain C2. Using a fluorescence lifetime-based actin-binding assay, we determined that all 3 HCM mutations increased in vitro binding affinity for actin in both unphosphorylated and phosphorylated C0-C2. We also found that the root mean square fluctuation (RMSF) values used to measure the in silico mobility of the protein in the trajectories (a total of 4 ms each) at either 25 or >50 ^{o}C induced moderate local structural changes in P161S and P371R and allosteric structural changes in Y237S as compared to immunoglobulin-like wild type C1 or C2 domain structures. In addition, mutations reduced in vitro protein folding stability of C0-C2, C1, and C2 relative to wild type, as expected, using differential scanning calorimetry (DSC) and protein solubility assays. These in vitro and in silico results suggest that structural changes in mutant cMyBP-C domains can cause altered myofilament binding, and thereby lead to dysfunction and HCM disease.

8-POS Board 8

TRUNCATED TITIN INCORPORATES INTO DCM PATIENT MYOCARDIAL SARCOMERE AND TRANSMITS FORCE ACROSS THE HALF-SARCOMERE

Quentin McAfee[1]; Matt Caporizzo[2]; Keita Uchida[2]; Kenneth C Bedi, Jr.[1]; Kenneth B Margulies[1]; Zolt Arany[1]; Benjamin Prosser[2];
[1]University of Pennsylvania, Cardiovascular Institute, Philadelphia, PA, USA
[2]University of Pennsylvania, Department of Physiology, Philadelphia, PA, USA

Truncated titin protein (TTNtv) has recently been shown by multiple groups to be present in the myocardium of patients with dilated cardiomyopathy caused by heterozygous truncations in TTN. However, it has remained unclear whether truncated titin incorporates into the myocardial sarcomere, possibly acting in a dominant negative fashion. To specifically detect truncated titin in the human $TTNtv^{+}$ DCM myocardium, we raised an antibody against a patient specific frameshift antigen, a proteome-unique sequence of amino acids appended to the C-terminus of a single patient's A-band truncation site. By western blot, this antibody specifically detects truncated titin at the expected molecular weight. Staining skinned cardiomyocytes from this patient with this antibody yields twin stripes 240nm from the M line, a location predicted by the truncation site, demonstrating truncated titin binds to the thick filament. To determine whether truncated titin was able to integrate with the Z-disc and transmit force across the stretched sarcomere, we stretched skinned cardiomyocyte fragments from this patient that were labeled with this antibody. When stretched from normal through supraphysiological sarcomere lengths, truncated titin remained attached to the thick filament. To determine if truncated titin was attached to the Z-disc and capable of transmitting force across the half-sarcomere, we treated stretched sarcomeres bearing TTNtv with 400mM KCl to disrupt the thick filament. This caused truncated titin to detach from the thick filament and retract to the Z-disk, consistent with titin's behavior as an entropic spring attached at one end to the Z-disk. These data demonstrate that truncated titin is incorporated into the myocardial sarcomere, and in binding to both the Z-disk and thick filament, transmits force across the half sarcomere. Broadly, these data support the hypothesis that truncated titin may act at the sarcomere in a dominant negative mechanism to promote the development of dilated cardiomyopathy.

11-POS Board 11

SKELETAL MYOSIN INHIBITOR EDG-4131 EXHIBITS A DISTINCT MECHANISM FOR MODULATING MUSCLE FUNCTION

Sarah J Lehman[1]; Weikang Ma[2]; Mike DuVall[1]; Benjamin Barthel[1]; Molly R Madden[1]; Marc Evanchik[1]; Thomas C Irving[2]; Alan J Russell[1];
[1]Edgewise Therapeutics, Boulder, CO, USA
[2] Illinois Institute of Technology, Center for Synchrotron Radiation Research and Instrumentation, Chicago, IL, USA

Skeletal myopathies are genetic diseases that lead to progressive muscle degeneration, leading to weakness and loss of ambulation. While etiologies vary, many types of myopathies are characterized by contraction-induced injury. Small molecules that limit sarcomeric contraction have shown slowing of skeletal muscle disease progression in animal models. One class of molecules targets myosin, inhibiting the enzymatic function of the motor protein and/or sequestering myosin in a state unable to bind actin, leading to reduced muscle contractility. EDG-4131 inhibited the ATPase activity in rabbit psoas myosin S1 (IC_{50}=1.1μM) and force production in fast skeletal muscle fibers. Further biochemical assessment of the super-relaxed state of rabbit psoas heavy meromyosin showed that EDG-4131 significantly increased the super-relaxed state (36μM EDG-4131: 60 ± 10% vs. DMSO: 72 ± 4%, p=0.02), reducing the number of available myosin heads for interaction with actin. Interestingly, X-ray diffraction patterns in rat EDL muscle showed that, in contrast to other myosin inhibitors such as blebbistatin that order the myosin heads close to thick filament backbone, EDG-4131 shifted the myosin heads towards actin (I_1/I_0: EDG-4131 = 0.28 vs Ctrl = 0.36) accompanied with a loss of helical ordering of the myosin heads under resting conditions (I_{M3}: EDG-4131 = 1.83 vs Ctrl = 1.31, I_{MLL1}: EDG-4131 = 1.46 vs Ctrl = 0.80). These data suggest that EDG-4131 does not inhibit myosin function by stabilizing the folded-back state of myosin heads but may lead to an undefined structural state of myosin that results in slowed ATP turnover and reduces lattice spacing below the optimal contractile range.

14-POS Board 14

MOLECULAR DYNAMICS SIMULATIONS REVEAL FUNCTIONAL CHANGES IN TROPONIN RESULTING FROM MUTATIONS IMPLICATED IN CARDIOMYOPATHIES

Sage A Malingen[1,2]; Matthew C Childers[1,2]; Travis Tune[2,3]; Kerry Kao[1,2]; Thomas Daniel[2,3]; Farid Moussavi-Harami[2,4,5]; Michael Regnier[1,2];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Center for Translational Muscle Research, Seattle, WA, USA
[3]University of Washington, Biology, Seattle, WA, USA
[4]University of Washington, Laboratory Medicine & Pathology, Seattle, WA, USA
[5]University of Washington, Division of Cardiology, Seattle, WA, USA

Sarcomeric proteins function in concert to power muscle contraction, but mutations to individual components can cause organ-scale dysfunction, such as hypertrophic and dilated cardiomyopathies. Here we consider troponin, which regulates the formation of actomyosin cross-bridges via a calcium-mediated shape change that leads to thin filament activation. We have used molecular dynamics simulations to elucidate how troponin's structure changes for a series of mutations in troponin C implicated in hypertrophic and dilated cardiomyopathies. By simulating all three subunits of the troponin complex, we retain the connected structure of the protein, allowing the impact of a point mutation to propagate throughout the protein. These models underscore that impairment in calcium binding and alterations in TnI's interaction with the hydrophobic patch of TnC lead to dysfunction. Further, we are deploying steered molecular dynamics to inform how mutations in the N lobe of cardiac troponin C alter calcium affinity and the interaction of the switch peptide with the hydrophobic patch. Ultimately, these steered molecular dynamics results can inform a spatially explicit model of the half sarcomere to predict how twitches change when troponin is mutated, bridging between the molecular scale and the emergent behavior of the half sarcomere.

17-POS Board 17

HYPERTROPHIC CARDIOMYOPATHY MUTATION R403Q DEVELOPS ACTIN-MEDIATED MECHANICAL AND STRUCTURAL DYSFUNCTION IN PORCINE VENTRICLE TISSUE

Saffie Mohran[1,3]; Matthew Childers[1,3]; Kristina Kooiker[2,3]; Jing Zhao[4]; Timothy McMillen[3]; Christian Mandrycky[1,3]; Stephanie Neys[2]; Jingyuan Yu[4]; Farid Moussavi-Harami[2,3]; Michael Geeves[5]; Thomas Irving[6]; Weikang Ma[6]; Michael Regnier[1,3];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Cardiology, Seattle, WA, USA
[3]University of Washington, Center for Translational Muscle Research, Seattle, WA, USA
[4]Dalian Medical University, College of Basic Medical Sciences, Dalian, China
[5]University of Kent, School of Biosciences, Canterbury, United Kingdom
[6]Illinois Institute of Technology, Biology, Chicago, IL, USA

Missense mutations in human β-cardiac myosin were first reported to cause malignant hypertrophic cardiomyopathy with the discovery of R403Q. Located in the upper 50KDa cardiomyopathy loop of myosin, there are several reports on the impact of R403Q on myosin structure, sarcomere contractile properties, and ATPase activity. Conflicting findings in contractile kinetics between human-patient samples and a transgenic rabbit model motivated us to study a novel MYH7 R403Q porcine model. Preliminary contractile experiments comparing control and R403Q demembranated ventricle strips showed R403Q expresses significantly faster k_{TR} (1.18s-1 vs 1.89s-1), greater Ca^{2+} sensitivity (pCa_{50} = 5.72 vs 5.89), and a decreased Hill coefficient (4.96 vs 2.57). Small angle x-ray diffraction on R403Q tissues demonstrated greater myosin disorder with decreased intensities in the myosin-based reflections (I_{MLL1} = 6.30 vs 0.69, I_{M3} = 9.35 vs 3.56) compared to WT cardiac muscle. Interestingly, R403Q tissue had blunted structural changes in response to dATP, a myosin activator, while the structural changes in response to mavacamten, a myosin inhibitor, were increased. These structural changes suggest that R403Q increases the population of ON myosin heads, a potential mechanism of hyper-contractility. To understand the direct impact of R403Q on myosin head structure in the absence of actin, we performed molecular dynamic (MD) simulations of the pre-power stroke state (M.ADP.Pi). Simulations showed the R403Q mutation primarily altered the surface area and electrostatic potential of myosin's actin binding surface. To complement the MD simulations, we isolated full-length myosin and performed stop-flow measurements of ATP-binding and single nucleotide turnover utilizing Mant-ATP. These actin-absent experiments showed no difference in ATP-binding and single nucleotide turnover kinetics between control and R403Q myosin. Future experiments will utilize HMM in the absence and presence of actin to assess ADP dissociation and actin-mediated ATP-binding.

20-POS Board 20

ASSESSING THE IMPACT OF HYPERTROPHIC CARDIOMYOPATHY-ASSOCIATED MISSENSE VARIANTS ON ALPHA-ACTININ 2 STRUCTURE/FUNCTION

Maya Noureddine[1]; Fiyaz Mohammed[2]; Katja Gehmlich[1];
[1]University of Birmingham, Institute of Cardiovascular Sciences, Birmingham, United Kingdom
[2]University of Birmingham, Institute of Immunology and Immunotherapy, Birmingham, United Kingdom

Alpha-actinin 2 (ACTN2) is a key protein at the Z-disk of the sarcomere. It is critical for stabilizing the contractile muscle apparatus and organizing the thin actin filaments. ACTN2 encompasses several structural domains, including an N-terminal actin-binding domain, a flexible neck region, a central rod domain of spectrin repeats, and two C-terminal EF-hand motifs. The functionally relevant ACTN2 dimer predominantly assembles via the central rod domain. Stabilization of the ACTN2 dimer interface also involves key residues in the neck region and EF34-hand motif. Genetic missense variants in ACNT2 are associated with inherited cardiac conditions, cardiomyopathies. In particular, they can cause hypertrophic cardiomyopathy (HCM), a genetic disease characterized by sudden cardiac death, left ventricular hypertrophy and diastolic dysfunction, but disease mechanisms are only poorly understood. Therefore, defining the molecular mechanisms by which ACTN2 variants lead to HCM may help to develop targeted therapeutic approaches. Herein, we examine the impact of 20 HCM-causing variants, identified from the human gene mutation database, on the ACTN2 structure/function using various modelling software packages, including I-TASSER, Phyre2, and AlphaFold2. We demonstrate that HCM-linked variants are distributed across most ACTN2 structural modules, except for the neck region. Based on structural predictions, two variants are predicted to destabilize the dimer interface. In addition, we identified different variants which could adversely impact the structural integrity of specific ACTN2 domains. Finally, we assessed which of the HCM-associated variants could impact ACTN2 ligand binding to actin, PIP2, titin, and CamKII. Collectively, this study suggests diverse molecular mechanisms by which individual genetic variants could adversely impact ACNT2 structure/function, leading to cardiomyopathies. It formulates hypotheses to be tested by wet-lab experiments, and may thereby be the first step towards the development of novel therapeutic strategies.

23-POS Board 23

HCM-CAUSING MUTATIONS G256E AND G768R IN B-CARDIAC MYOSIN CAUSE HYPERCONTRACTILITY BY OPENING MYOSIN HEADS

Divya Pathak[1,2]; Aminah Dawood[1]; Kathleen Ruppel[1,2]; James A Spudich[1,2];
[1]Stanford University, Biochemistry, Stanford, CA, USA
[2]Stanford Cardiovascular Institute, Stanford, CA, USA

Hypertrophic cardiomyopathy (HCM) is an inherited cardiovascular disease that affects up to 1 in 200 people and is the leading cause of sudden cardiac death among the young. HCM is marked by hypercontractility, abnormal thickening of the heart muscle, cardiac hypertrophy and fibrosis. Over 30% of all known HCM mutations can be found in the sarcomere protein β-cardiac myosin, but their molecular mechanism remains poorly understood. β-cardiac myosin is a large, highly allosteric protein, making it challenging to predict functional changes due to mutations. Studies over the past decade have shown that myosin samples two conformational states– an 'open' state, where the myosin heads are available to interact with actin and a 'closed' state, where the heads fold back onto the myosin tail and are no longer available to interact with actin. We have previously shown that several HCM mutations cause hypercontractility by increasing the myosin heads in the 'open' state. This study focuses on two point mutations in distinct regions of the myosin motor domain – G256E and G768R. G256 is located in the hairpin turn in the myosin transducer region bordering the myosin mesa, whereas G768 is present at the end of its converter in the motor domain of β-cardiac myosin. Both mutations have been predicted to destabilize the 'closed' or folded-back state. I will be presenting the characterization of these mutations using single-molecule and ensemble assays. Although the velocity for G256E decreased by ~20%, the mutation increased the number of myosin in 'open' state, resulting in hypercontractility. Interestingly, G768R led to a steep decrease of ~80% in actin velocity in an in vitro actin gliding assay. On a single molecule level, this mutation significantly decreased myosin's detachment rate without altering force sensitivity. Despite decreasing the velocity and ATPase activity per motor head, I will present results showing how the mutation compensates for the reduced ATPase activity by biasing the myosin equilibrium towards the 'open state'.

26-POS Board 26

QUANTIFYING AND MECHANOSIGNALING IN HIPSC-CARDIOMYOCYTES IN HEALTH AND DISEASE

Beth Pruitt;
[1]UCSB, BioE, Santa Barbara, CA, USA

We combine microfabrication to control the ligands, stiffness, and morphology of biointerfaces to enable quantitative measurements of structure and mechanical function of cells through video analysis of cells patterned on microfabricated traction force microscopy devices. We use this workflow to manipulate and measure the mechanobiology of single cells to study maturation, remodeling, and functional changes of muscle cells in response to mechanical challenges, pharmacological treatments, or disease mutations. For example, we have used our platform to study Hypertrophic Cardiomyopathy (HCM) which is associated with thickening of the left ventricular wall, hypercontractility, and remodeling of the heart that ultimately reduces ventricular volume and pumping efficiency. HCM is characterized by changes in cellular level genotype and phenotype in the motor unit of the heart – the striated cardiac muscle cells known as cardiomyocytes. Using single cell mechanobiology studies, we examine how the effects of single point mutations propagate to change the contractile dynamics and cellular morphology (sarcomere spacing, spread area, myofibril alignment) of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). We micropattern islands of adhesive protein to constraining the spreading and alignment of hiPSC-CM on hydrogel substrates containing fluorescent microbeads as fiducial markers for traction force microscopy (TFM). We deploy substrate stiffnesses ranging from physiological (10 kPa) to heavily diseased/fibrotic (100 kPa) to test the role of increased “afterload” in functional phenotypes. We use image and video analysis to assess the contractile dynamics of the hiPSC-CM in terms of force, power, and velocities of relaxation and contraction.

AN EXPERIMENTAL APPROACH TO MANIPULATE THE MULTI-SCALE COMPONENTS OF WHOLE-BODY MOVEMENT THAT INFLUENCE SINGLE MUSCLE FIBER WORK AND POWER OUTPUT

David C Lin[1,2]; **Bertrand C.W. Tanner**[1,2];
[1]Washington State University, Integrative Physiology and Neuroscience, Pullman, WA, USA
[2]Washington State University, Voiland School of Chemical Engineering and Bioengineering, Pullman, WA, USA

Locomotion includes coupling between muscles, tendons, bones, and joints, which intrinsically form a multi-scale mechanical feedback system. Namely, the forces and length changes that occur in a muscle fiber are influenced by muscle architecture, mechanical characteristics of the attached tendon, musculoskeletal geometry, and inertias of the limbs and body. To probe how muscle performance is affected by these elements, it is necessary to manipulate the properties of these elements to better understand system coupling. While this manipulation is possible in computational models, experimentally manipulating these elements is difficult, especially as the scale becomes larger and the system contains more elements. To address this challenge, we have developed a virtual mechanical load environment for single skeletal muscle fibers based on the concept of mechanical impedance and implemented by force-feedback. Within this environment, we can manipulate crossbridge mechanisms via solution-based methods, as well as muscle architecture, tendon, and inertial properties. These manipulations can be either parametric (e.g., increasing tendon compliance) or introduce nonlinear behavior (e.g., including the “toe” region of tendon). Our initial study addressed how tendon compliance influences muscle power and work output as a fiber is activated from rest (relaxed conditions). We tested compliance values spanning a 50-fold change, while activating chemically-skinned fibers from a rat medial gastrocnemius. Power output from the fiber increased nonlinearly with increases in tendon compliance. Interestingly, there was not a large change in muscle force throughout the movement for the different compliances, implying force-velocity effects were not prominent, potentially due to the activation-driven force increase from rest. Future studies will examine how the toe region of tendon and rotation of fibers within a whole muscle (i.e., changes in pennation angle) influence muscle power generation.

ASSESSING THE IMPACTS OF SMALL VARIANTS IN THE GIANT SARCOMERIC PROTEIN TITIN ASSOCIATED WITH MUSCLE DISEASE

Timir Weston[1]; Franca Fraternali[2];
[1]King's College London, London, United Kingdom
[2]University College London, London, United Kingdom

Giant modular sarcomeric proteins such as titin have come under increasing scrutiny for their contributions to diseases such as skeletal and cardiac myopathies. Many variants in titin have been linked with these conditions through computational and experimental studies, highlighting that even relatively small, individual missense mutations may have far-reaching structural and functional consequences. However, assessing the impact of all possible single nucleotide variants (SNVs) in titin is complicated by the very large size of this protein. To aid in this task, we have updated the centralised resource for information relating to titin variants, TITINdb2, with newly described pathogenic variants, annotations, structural models, and pathogenicity predictions. By combining these various data together, we can explore the mutational landscape of titin and improve the accuracy of annotation of variant impacts for titin missense variants. We demonstrate the potential of more computationally expensive features, using molecular dynamics, in adding information lacking from simpler representations of these proteins based on sequence and protein structure. We further show how a variant classifier trained on these features improves on the ability to discriminate between neutral and deleterious variants. These offer a first step towards improving our understanding of titin's function and dysfunction in relation to the development of skeletal and cardiac myopathies. Giant modular sarcomeric proteins such as titin have come under increasing scrutiny for their contributions to diseases such as skeletal and cardiac myopathies. Many variants in titin have been linked with these conditions through computational and experimental studies, highlighting that even relatively small, individual missense mutations may have far-reaching structural and functional consequences. However, assessing the impact of all possible single nucleotide variants (SNVs) in titin is complicated by the very large size of this protein. To aid in this task, we have updated the centralised resource for information relating to titin variants, TITINdb2, with newly described pathogenic variants, annotations, structural models, and pathogenicity predictions. By combining these various data together, we can explore the mutational landscape of titin and improve the accuracy of annotation of variant impacts for titin missense variants. We demonstrate the potential of more computationally expensive features, using molecular dynamics, in adding information lacking from simpler representations of these proteins based on sequence and protein structure. We further show how a variant classifier trained on these features improves on the ability to discriminate between neutral and deleterious variants. These offer a first step towards improving our understanding of titin's function and dysfunction in relation to the development of skeletal and cardiac myopathies.

Wednesday, July 19
POSTER SESSION III
16:30 - 18:00
Sibson Foyer

All posters are available for viewing during all poster sessions, however, below are the formal presentations for Wednesday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Odd-Numbered Boards 16:30 – 17:15 | Even-Numbered Boards 17:15 – 18:00

Chalovich, Joseph	**3 – POS**	**Board 3**
Ehler, Elisabeth	**6 – POS**	**Board 6**
Irving, Thomas	**9 – POS**	**Board 9**
Lehman, Sarah	**12 – POS**	**Board 12**
Mandrycky, Christian	**15 – POS**	**Board 15**
Naim, Ateeqa	**18 – POS**	**Board 18**
Palmer, Bradley	**21 – POS**	**Board 21**
Pilagov, Matvey	**24 – POS**	**Board 24**
Radocaj, Ante	**27 – POS**	**Board 27**
Teitgen, Abigail	**30 – POS**	**Board 30**
White, Howard	**33 – POS**	**Board 33**

Posters should be set up on the morning of Monday, July 17, and removed by noon on Thursday, July 20. All uncollected posters will be discarded.

3-POS Board 3

THE UNSTRUCTURED C-TERMINAL REGION OF TROPONIN T RETARDS CALCIUM ACTIVATION OF STRIATED MUSCLE

Joseph M Chalovich[1]; Li Zhu[1];
[1]Brody School of Medicine ECU, Biochem & Mol Biol, Greenville, NC 27834, NC, USA

Calcium binding to TnC alters contacts among troponin subunits leading to reorientation of tropomyosin on actin and a large increase in activity. Eisenberg and Weihing (10.1038/2281092a0) were the first of many to show that myosin lacking bound ATP or ADP-Pi (activating states) can further increase activity even at saturating calcium. That is, something in the thin filament retards full calcium activation. That limitation of activation requires basic residues within the C-terminal 14-16 residues of TnT. Removal of those residues from TnT doubles the ATPase activity. Negative charges introduced at some positions in that region of TnT are also activating. Fluorescence and kinetics measurements show that the unstructured C-terminal basic region of TnT stabilizes the inactive state of regulated actin formed in the absence of calcium and "activating" species of myosin (see 10.1016/S0006-3495(81)84777-7 for activating species) and destabilizes the active state at high calcium levels. Furthermore, the C-terminal basic region of TnT increases the calcium level required for activation of skeletal (10.1021/acs.biochem.0c00499) and cardiac (10.1021/acs.biochem.0c00430) fibers. A FRET analysis showed that calcium binding positioned the C-terminal region of TnT, and the inhibitory and switch regions of TnI away from actin-tropomyosin (10.1021/acs.biochem.2c00090). Unlike those regions of TnI, the C-terminal region of TnT did not appear to contact TnC, although it moved toward it. Removal of the charged residues from C-terminal TnT mimicked the calcium induced changes in the position of C-terminal TnT and the inhibitory region of TnI. However, the switch region of TnI was not near TnC as it was in the calcium state. Conclusion: The disordered, basic, C-terminal region of TnT is necessary for full inactivation, it inhibits activation by Ca^{2+} and permits full activation upon binding activating forms of myosin to actin. It does all of this in concert with the other known elements of troponin.

6-POS Board 6

MISSENSE MUTATIONS IN THE CENTRAL PART OF CARDIAC MYBP-C AND THEIR POTENTIAL CONTRIBUTION TO HYPERTROPHIC CARDIOMYOPATHY

Amy Pearce[1]; Saraswathi Ponnam[2]; Mark R Holt[1]; Thomas Randall[1]; Thomas Kampourakis[2]; **Elisabeth Ehler**[1,2];
[1]King's College London, School of Cardiovascular and Metabolic Medicine and Sciences, London, United Kingdom
[2]King's College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom

Mutations in the MYBPC3 gene are amongst frequent causes for hypertrophic cardiomyopathy. In addition to truncating mutations, there also missense mutations were reported, which tend to be found in the central domains of the MyBP-C molecule. This suggests that these central domains are more than just a spacer between the better characterised N- and C-terminal domains. We have analysed the potential impact of four different missense mutations (E542Q; G596R; N755K; R820Q) that are spread over the domains C3 to C6 on the function of MyBP-C in vitro and in cardiomyocytes. Effect on domain stability, interaction with thin filaments, binding to myosin and subcellular localisation behaviour were assessed. Our studies show that these missense mutations result in slightly different phenotypes at the molecular level that are mutation specific, but that the expected functional readout of each mutation provides a valid explanation for an impaired contribution of MyBP-C to the regulation of muscle contraction.

9-POS Board 9

INTERPRETING EQUATORIAL X-RAY DIFFRACTION PATTERNS FROM STRIATED MUSCLE WITH MULTISCALE MODELING

Momcilo Prodanovic[1]; Yiwei Wang[3]; Srboljub Mijailovich[2]; **Thomas C. Irving**[3];
[1]University of Kragujevac, Institute for Information Technologies, Kragujevac, Serbia
[2]FilamenTech, Inc, Newton, MA, USA
[3]Illinois Insitute of Technology, Biology, Chicago, IL, USA

Synchrotron small-angle X-ray diffraction is the method of choice for nm-scale structural studies of striated muscle under physiological conditions and on millisecond time-scales. The lack of generally applicable computational tools for modeling X-ray diffraction patterns from intact muscles have been a significant barrier to exploiting the full potential of this technique. Here we report a novel, "forward problem" approach using the spatially explicit computational simulation platform, MUSICO, to predict equatorial small angle X-ray diffraction patterns and the force output simultaneously from resting and isometrically contracting rat skeletal muscle that can be compared to experimental data. The simulation generates families of thick-thin filament repeating units each with their individually predicted occupancies of different populations of active and inactive myosin heads that can be used to generate 2D projected electron density models based on known Protein Data Bank structures. Using this approach, we found that we could recapitulate 7 independent experimental intensities (out to the 4,0 reflection) from relaxed rat soleus and EDL muscle by varying only the proportion of heads in the parked state and a temperature factor type disorder term. Fits to contracting data could be achieved by adjusting only the temperature factor term and the number of force-producing crossbridges predicted by MUSICO to match the force levels observed experimentally. The developments presented here demonstrate the feasibility of combining X-ray diffraction and spatially explicit modeling to form a powerful hypothesis generating tool that can be used to motivate experiments that can reveal emergent properties of muscle. Supported by NIH and the AHA.

12-POS Board 12

POINT MUTATIONS AT THE SAME RESIDUE IN BETA MYOSIN HEAVY CHAIN LEAD TO DISTINCT DISEASES AND MOLECULAR PHENOTYPES

Sarah J Lehman[1]; Artur Meller[2]; Jeffrey M Lotthammer[2]; Shahlo O Solieva[4]; Stephen J Langer[1]; Michael J Greenberg[2]; Jil C Tardiff[3]; Gregory R Bowman[4]; Leslie A Leinwand[1];
[1]University of Colorado, Molecular, Cellular, and Developmental Biology, Boulder, CO, USA
[2]Washington University, Department of Biochemistry and Molecular Biophysics, St. Louis, MO, USA
[3]University of Arizona, Department of Biomedical Engineering, Tucson, AZ, USA
[4]University of Pennsylvania, Department of Biochemistry and Biophysics, Philadelphia, PA, USA

A frequently described phenomenon in genetic cardiomyopathies is how similar mutations in a single protein can lead to distinct clinical phenotypes. One example is described by two missense mutations in β-myosin heavy chain (β-MyHC) that have been linked to hypertrophic cardiomyopathy (HCM) (Ile467Val, I467V) and left ventricular non-compaction (LVNC) (Ile467Thr, I467T). To investigate how these mutations lead to different pathologies, we studied the molecular effects of each mutation using recombinant human β-MyHC Subfragment 1 (S1) in in vitro assays. LVNC-I467T S1 exhibited similar chemomechanical function to the HCM-I467V S1 mutation, including unchanged ATPase activity (1.7±0.2/second, 1.7±0.3/second, β-WT S1: 1.7±0.1/second) and enhanced actin velocity (1.4±0.1/second, 2.5±0.3/second, β-WT S1: 3.0±0.2/second). LVNC-I467T showed a significant increase in the super-relaxed (SRX) state of myosin (93±4%) compared to both WT and the HCM-I467V mutant (16±4%, 10±2%, respectively). We conducted molecular dynamics simulations to explore how the LVNC-I467T mutation affects myosin dynamics in the pre-powerstroke state. Our simulations suggest I467T allosterically disrupts interactions between ADP and the nucleotide-binding pocket by breaking bonds between residues that stabilize ADP in the active site, leading to a weaker binding and a predicted increase in ADP release rate. This predicted ADP increased release rate may underlie the enhanced actin velocity measured in LVNC-I467T. Moreover, the unchanged ATPase activity of LVNC-I467T likely results from increased ADP release coupled to stabilization of the SRX state of myosin. This uncoupled chemomechanical function may initiate contractile dysregulation that triggers a distinct signaling pathway that progresses the LVNC-like phenotype. Alternatively, HCM-I467V variant exhibited a more predicted “gain of function” phenotype. Together, our analyses suggest
that phenotypic complexity originates at the molecular level and is critical to understand disease progression and develop therapies.

15-POS Board 15

EMBRYONIC MYOSIN MUTATIONS ASSOCIATED WITH DISTAL ARTHROGRYPOSIS ALTER THE MECHANICS AND MATURATION OF HIPSC DERIVED SKELETAL MUSCLE

Christian Mandrycky[1]; Saffie Mohran[1]; Matthew Childers[1]; Shawn Luttrell[3]; Elizabeth Choi[1]; Kati Buckingham[2]; Michael Bamshad[2]; David Mack[3]; Michael Regnier[1];
[1]University of Washington, Bioengineering, Seattle, WA, USA
[2]University of Washington, Pediatrics, Seattle, WA, USA
[3]University of Washington, Rehabilitation Medicine, Seattle, WA, USA

Distal arthrogryposis (DA) is a skeletal muscle disorder characterized by joint contractures predominantly localized in the distal extremities. DA associated syndromes like Freeman-Sheldon Syndrome (FSS) are linked to mutations in the MYH3 gene that encodes the embryonic skeletal muscle myosin. To study the mutation and its effect on developing muscle, we generated human induced pluripotent stem cell (hiPSC) lines bearing T178I or R672C MYH3 mutations. hiPSC were differentiated into skeletal muscle and evaluated for differences in the maturation of the contractile unit and its functional performance. R672C mutations in MYH3 were associated with alterations in myosin isoform content, with homozygous R672C having reduced MYH3 levels in day 7 cells and elevated levels of MYH7 relative to isogenic control. Preliminary myofibril mechanics measurements showed T178I myotubes generated significantly greater specific force compared to control myofibrils. Diseased myofibrils also expressed significantly slower rates of relaxation and prolonged thin filament deactivation compared to control myofibrils. Myofibril preparations were also used to compare ATP binding rates across conditions. Preliminary results showed homozygous R672C myofibrils have faster ATP binding rates compared to heterozygous and control preparations. Molecular dynamics simulations of R672C and T178I mutations in the post rigor state showed greater separation between the B-sheet and SH-Helix than in controls. Mutation also disrupted local salt bridges and hydrogen bond formation in some residues that help stabilize the nucleotide binding pocket and converter-connected helix. Interrupted structural communication between the nucleotide binding pocket and surrounding functional regions may underly the impaired relaxation phenotype. Ongoing studies will further characterize the effect of mutation on actin-myosin binding, crossbridge cycling kinetics, the maturation of skeletal myotubes over time, and myosin isoform switching dynamics.

18-POS Board 18

INVESTIGATING THE SPATIAL DISTRIBUTION OF SRX IN CARDIAC MYOFIBRILS USING SINGLE MOLECULE IMAGING

Ateeqa Naim[1]; Matvey Pilagov[1]; Sonette Steczina[2]; Michael Regnier[2]; Michael Geeves[1]; Neil Kad[1];
[1]University of Kent, School of Biosciences, Canterbury, United Kingdom
[2]University of Washington, Bioengineering, Seattle, WA, USA

Contraction of striated cardiac muscle results from interactions between actin from the thin filament and myosin from the thick filament. The availability of individual myosin heads for these interactions is dependent on the state they occupy; these states are disordered relaxed (DRX – able to interact with actin) or super-relaxed (SRX – does not interact with actin). To develop a full understanding of dual filament regulation, the distribution of these states across the thick filament needs to be known, and how they are controlled needs to be understood. Studying β-cardiac myosin of the porcine left ventricle, we have been able to assign the activity of individual myosin via single molecule imaging of fluorescently tagged ATP. We determined that 51.5% of myosin in the C-zone occupy the SRX state, while in the D-zone this is 46.1%. Overall, 44.3% of β-cardiac myosin occupies the SRX state. Crucially we are focusing on the α-cardiac myosin dominated porcine left atria to understand whether in addition to the faster ATPase cycling rate, the amplitude and zonal distribution of the myosin states vary from that of β-cardiac. In addition, we are examining the distribution of SRX vs DRX using much higher spatial resolution to provide a high resolution heatmap of activity across the whole thick filament.

21-POS Board 21

VALIDATION OF NOVEL INTERPRETATION OF FORCE RESPONSE OF CARDIAC MUSCLE TO QUICK STRETCH BY COMPUTER SIMULATIONS

Bradley M Palmer[1];
[1]University of Vermont, Molecular Physiology and Biophysics, Burlington, VT, USA

The force response of demembranated mammalian cardiac muscle after a quick stretch is typically interpreted to represent the initial stretch of attached myosin crossbridges (phase 1), detachment of stretched crossbridges at an exponential rate (phase 2) followed by crossbridges reattaching in increased numbers due to length-dependent activation (phases 3 and 4). We propose that phase 2 additionally reflects an enhanced detachment rate of myosin crossbridges due to strain on the myosin crossbridge thereby explaining the nadir of the force response often observed below the original isometric force. To test this idea further, we used a computer model of crossbridge kinetics with attachment and detachment governed by Monte-Carlo methods. Unitary force was assigned when a crossbridge was attached, and an elastic force was generated when an attached crossbridge was displaced. The duration of detached crossbridge was governed by a first-order attachment rate f_0, and the duration of attached crossbridge displaced a distance x was governed by the detachment rate $g(x) = g_0 + g_1 x$, where g_0 is the detachment rate with no displacement and g_1 indicates the sensitivity to displacement. An analytical solution suggested the exponential decay rate of phase 2 represents ($f_0 + g_0$) and the exponential rise rate of phase 3 represents g_0. The depth of the nadir between phases 2 and 3 is proportional to g_1 and inversely proportional to g_0. The computer simulations demonstrated that this interpretation is correct if the mechanical response is limited to a behavior displaying the homogeneous and additive properties of a linear system and if the myosin kinetics are reasonably represented by two states. This scheme for interpreting the force response to a quick stretch suggests that myosin detachment rate is enhanced with stretch and needs to be considered as contributing to the mechanical response of striated muscle subjected to length perturbations.

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SUPER-RESOLUTION SINGLE-MOLECULE IMAGING OF ATP USAGE IN MYOFIBRILS TO STUDY THICK FILAMENT REGULATION

Matvey Pilagov[1]; Sonette Steczina[2]; Ateeqa Niam[1]; Michael Regnier[2]; Michael A Geeves[1]; Neil M Kad[1];
[1]University of Kent, School of Biological Sciences, Canterbury, United Kingdom
[2]University of Washington, Department of Bioengineering, Seattle, WA, USA

Controlling access of myosin to actin in muscle modulates how energy is used. This control occurs at both the thin and thick filaments levels; with the latter involving two states of myosin: disordered relaxed (DRX – able to interact with actin), and super-relaxed (SRX – does not interact with actin). The amount of DRX defines the number of myosins available to interact with actin. However, it is still uncertain how this SRX-DRX equilibrium is distributed within the sarcomere. By fluorescently labelling ATP, we are able to measure the activity of individual myosins within myofibrils. Super-resolution deconvolution provides ~30 nm localization of these activities, enabling assignment to the three zones of the thick filament P, C and D. For fast skeletal muscle (rabbit psoas), we observed 53% of all heads in the C-zone were SRX, compared with 35% and 44% in the P- and D-zones, respectively. This suggests myosin binding protein C (MyBP-C) influences the SRX-DRX equilibrium. To investigate if phosphorylation of MyBP-C affects the population of SRX, we treated myofibrils with protein kinase A (PKA). We found that PKA decreased SRX to 34% in the C-zone, whereas the P- and D-zones were not affected. Nowhere is the control of myosin head accessibility more relevant than in the heart, therefore we have studied the localization of DRX and SRX in porcine cardiac myofibrils, which possess human equivalent beta-cardiac myosin. We will report on the effects of PKA phosphorylation and the recently FDA-approved hypertrophic cardiomyopathy drug, mavacamten, in these myofibrils. In summary, our results directly show that PKA treatment of muscle releases some but not all myosin heads, equally mavacamten increases SRX but does not completely eradicate DRX. Therefore, the activation and repression of myosins within the sarcomere is a combination of multiple competing factors that require an in-depth understanding, especially relevant as we begin to develop drugs to modulate this equilibrium.

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MODEL OF TRANSCRIPTIONAL BURSTING EXPLAINS INTERCELLULAR VARIABILITY OBSERVED IN HYPERTROPHIC CARDIOMYOPATHY WITH MUTATIONS IN MYOSIN HEAVY CHAIN OR TROPONIN I

Ante Radocaj[1]; Valentin Burkart[1]; Kathrin Kowalski[1]; Judith Montag[1]; Theresia Kraft[1];
[1]Hannover Medical School, Institute for Molecular and Cell Physiology, Hannover, Germany

Hypertrophic cardiomyopathy (HCM) in most mutation-positive patients is caused by heterozygous mutations in sarcomeric proteins. We observed that HCM is often associated with an increased variability between cardiomyocytes, which is significantly larger than between cardiomyocytes from donors. This variability includes transcriptional activity, mRNA expression and contraction as well as morphological parameters. We hypothesize that the observed variability can be explained by transcription of both, mutated and wildtype alleles, occurring in independent stochastic bursts. We developed a numerical model based on the Euler method which entails (i) the polyploidy of cardiomyocytes, (ii) the stochastic burst-like activation of both alleles, (iii) the synthesis of pre-mRNA, (iv) the splicing of pre-mRNA to mRNA and mRNA degradation, and (v) the synthesis and degradation of the affected protein. We applied the model to two different heterozygous myosin mutations and one heterozygous troponin I mutation. The corresponding rate constants for mRNA and protein synthesis and degradations were taken from the literature. By fitting the distribution of ploidy and the rate constants for activation and inactivation of transcription and for the splicing of pre-mRNA to mRNA, we were able to closely reproduce the observed distributions of active transcription sites in the nuclei, of the absolute mRNA counts, of the mutant to wildtype mRNA ratio, and of the produced force at intermediate calcium concentrations. We conclude that for all three studied HCM mutations the observed phenotypical variations could be associated with the mechanism of stochastic transcriptional bursting.

2-DEOXY-ATP IMPROVES SYSTOLIC VENTRICULAR FUNCTION IN A MULTISCALE COMPUTATIONAL MODEL OF HEART FAILURE

Abigail E Teitgen[1]; Marcus Hock[1]; Kimberly J McCabe[2]; Matthew C Childers[3]; Gary Huber[4]; Daniel Beard[5]; Michael Regnier[3]; Andrew McCulloch[1];
[1]University of California, San Diego, Bioengineering, San Diego, CA, USA
[2]Simula Research Laboratory, Computational Physiology, Oslo, Norway
[3]University of Washington, Bioengineering, Seattle, WA, USA
[4]University of California, San Diego, Chemistry and Biochemistry, San Diego, CA, USA
[5]University of Michigan, Molecular and Integrative Physiology, Ann Arbor, MI, USA

2-deoxy-ATP (dATP), a candidate therapeutic for heart failure, improves cardiac contractility and lusitropy by acting on myosin to increase the rate of crossbridge binding and cycling, and by increasing the rate of calcium transient decay. However, the molecular mechanisms behind these effects and how observed therapeutic responses to dATP are achieved – even when it is only a small fraction of the total ATP pool – remain poorly understood, especially in models of heart failure, in which energy metabolism is impaired. We developed a novel multiscale computational modeling framework to address these questions. We conducted molecular dynamics (MD) and Brownian dynamics (BD) simulations to assess (d)ADP.Pi-myosin/actin association rates. We then utilized these association rates to constrain a sarcomere mechanics model, and combined this with experimental calcium transient data to simulate the effects of dATP at the myocyte level. We then utilized a model of rat biventricular mechanics, energetics, and circulation to predict the effects of elevated dATP at the tissue and ventricular scales. MD and BD simulations showed that dATP increases actomyosin association rate via stabilization of pre-powerstroke myosin. Model predictions indicated that dATP destabilizes the super-relaxed state of myosin, leading to large changes in force with small fractions of dATP. The integrative effects of dATP on improved calcium handling and increased crossbridge binding and cycling augmented myocyte contractility and lusitropy, and could fully explain increases in myocyte shortening and relaxation observed experimentally. In a failing heart model, we predicted improvements in left ventricular function with no additional impairment of metabolic state with only 1% dATP. This was due at least in part to improved energy efficiency with elevated dATP. This novel multiscale analysis has elucidated how molecular mechanisms of dATP lead to improved cardiovascular function in heart failure.

STRUCTURE OF PREPOWERSTROKE ACTOMYOSIN BY CRYOELECTRONMICROSCOPY AT 10 MS AND 5A

Howard D White[1,2]; Risi Cristina[1]; David Klebl[2]; Stephen Muench[2]; Charlie Scarff[2]; Vitold Galkin[1];
[1]Eastern Virginia Medical Center, Physiological Sciences, Norfolk, VA, USA
[2]Leeds University, Astbury Center, Leeds, United Kingdom

We have determined the structure of the actomyosin complex formed 10 ms and 120 ms after mixing myosin-ADP-Pi with f-actin. High-resolution studies of actomyosin have been limited to equilibrium conditions with either ADP bound or an empty active site and the structure of the short lived primed, prepowerstroke, structure of the catalytic cycle has previously remained elusive. Here, using millisecond time-resolved cryo-electron microscopy we have determined the structure of the prepowerstroke actomyosin complex formed 10 ms after mixing myosin-ADP-Pi with f-actin. The predominant structure at 10 ms reveals myosin binding the actin filament through its lower 50 kDa subdomain with only the loop2 interacting with the actin surface. The predominant structure 120 ms after mixing with actin is a second conformational state in which the actin-binding cleft is closed and the lever is in a post-power stroke position, similar to previous strongly-bound actomyosin structures. Together, the two states represent the start and end positions of the powerstroke and allow us to assemble the most complete picture of the actomyosin catalytic cycle to date.