View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector



determine whether myofilaments activated with ADP (without Ca) are sensitive to kinase treatment.
Results: We show that ADP, in the absence of Ca, accelerates cross-bridge cycling and force development in cardiomyocytes from HF patients, which was sensitive to PKA-mediated phosphorylation (i.e. decreased sensitivity to ADP). Ca-sensitivity increased in the presence of increasing [ADP] and was accompanied by significant slowing cross-bridge cycling kinetics. This was correlated with significant increases in residual force enhancement (i.e. high initial tension recovery).
Conclusions: The current data show that high [ADP] reduces the ability to desensitize myofilaments to Ca, which likely compromises restoration of end-diastolic length. High ADP increased cross-bridge strain (i.e. diastolic dysfunction) and depressed myofilament cycling kinetics, which may limit muscle shortening (i.e. systolic dysfunction). The present study suggests that inability to lower myocardial ADP levels can be a primary determinant of contractile dysfunction and disease progression in human HF.

### 1002-Plat
**Myocardial Strain Rate Modulates the Speed of Relaxation in Dynamically Loaded Twitch Contractions**

**Kenneth S. Campbell**, Charles S. Chung.
Physiology and Center for Muscle Biology, University of Kentucky, Lexington, KY, USA.

Slow myocardial relaxation is an important clinical problem in about 50% of patients who have heart failure. Prior experiments had suggested that the slow relaxation might be a consequence of high afterload (hypertension) but large clinical trials testing this hypothesis have failed; lowering blood pressure in patients who have heart failure with preserved ejection fraction does not help clinical outcomes. We performed new experiments using mouse, rat, and human trabeculae (Chung et al., Biophys J. 106,564a, 2014) and showed that it is not afterload but the strain rate at end systole that determines the subsequent speed of relaxation. To investigate the molecular mechanisms that drive this behavior, we ran simulations of our mechanical experiments using the freely available software MyoSim (http://www.myosim.org). This software simulates the mechanical properties of dynamically activated half-sarcomeres by extending A.F.Huxley's cross-bridge distribution technique with calcium activation and cooperative effects. We discovered that our experimental data could be reproduced using a relatively simple framework consisting of a single half-sarcomere pulling against a series elastic spring. Further analysis of the simulations suggested that quick stretches speed myocardial relaxation by detaching myosin heads and thereby disrupting the cooperative mechanisms that would otherwise prolong thin filament activation. The simulations therefore identify myofilament kinetics and tissue strain rate as potential therapeutic targets for heart failure attributed to slow relaxation.

### 1003-Plat
**Myosin MgADP Release Rate Decreases at Longer Sarcomere Length to Prolong Myosin Attachment in Skinned Rat Myocardial Strips**

**Bertrand C.W. Tanner**, Peter O. Awinda, Jason J. Breithaupt.
Integrative Physiology and Neuroscience, Washington State University, Pullman, WA, USA.

Cardiac contractility increases as sarcomere length (SL) increases, suggesting that intrinsic molecular mechanisms underlie the Frank-Starling relationship to confer increased cardiac output with greater ventricular filling. Myosin's capacity to generate force is $Ca^{2+}$-regulated by thin-filament proteins and sarcomere length, which dictates the number of potential actin-myosin cross-bridge interactions. One mechanism underlying greater cardiac contractility at longer SL could involve longer myosin attachment duration ($t_{on}$). To test this idea, we used stochastic length-perturbation analysis in skinned rat papillary muscle strips to measure $t_{on}$ as [MgATP] varied (0.05-5 mM) at 1.9 and 2.2 μm SL. From this $t_{on}$-MgATP relationship, we calculated cross-bridge MgADP release rate ($k_{-ADP}$) and MgATP binding rate ($k_{+ATP}$). As MgATP increased $t_{on}$ decreased hyperbolically for both SL, but $t_{on}$ was roughly 50% longer for 2.2 vs. 1.9 μm SL at each [MgATP] ($25\pm3$ vs. $16\pm1$ ms at 5 mM MgATP, 17° C, $p<0.05$). These $t_{on}$ differences arose from slower $k_{-ADP}$ at 2.2 μm SL ($42\pm3$ vs. $74\pm8$ $s^{-1}$, $p<0.001$), as MgATP binding rates did not differ with SL ($281\pm56$ vs. $327\pm93$ $mM^{-1}$ $s^{-1}$). Absolute tension values were greater at 2.2 vs. 1.9 μm SL for relaxed ($4.4\pm0.7$ vs. $0.8\pm0.2$ kPa at pCa 8.0, $p<0.001$) and maximally activated ($20.0\pm1.4$ vs. $14.2\pm1.6$ kPa at pCa 4.8, $p<0.001$) conditions, and the force-pCa relationship was more sensitive to $Ca^{2+}$ at 2.2 μm SL ($pCa_{50}=5.45\pm0.01$ vs. $5.36\pm0.01$, $p<0.05$). These increased tension values suggest that cross-bridges may bear greater loads at longer SL, which diminishes MgADP release to prolong $t_{on}$ and amplify cooperative cross-bridge contributions to thin filament activation. Therefore, load-dependent rates of the actomyosin cross-bridge cycle may vary with SL to contribute, in part, to the Frank-Starling relationship in the heart.

### 1004-Plat
**Inherent Force-Dependent Properties of β Cardiac Myosin Contribute to the Force-Velocity Relationship of Cardiac Muscle**

Michael J. Greenberg, Henry Shuman, **E. Michael Ostap**.
Pennsylvania Muscle Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.

The cardiac cycle is a tightly regulated process in which the heart generates power during systole and relaxes during diastole. Appropriate power must be generated to effectively pump blood against cardiac afterload. Dysfunction of this cycle has devastating consequences for affected individuals. Cardiac power output is regulated by several feedback mechanisms (e.g. neuronal, hormonal, mechanical) which ultimately lead to changes in the force and power output of the molecular motor, β-cardiac myosin (βCM). Despite its importance in driving and regulating cardiac power output, the effect of force on the contractility of a single βCM has not been measured at physiological [ATP]. Using optical trapping techniques, we found that similar to some other myosins, βCM has a two-substep working stroke where the second mechanical substep is associated with ADP release. At saturating [ATP] (4 mM), forces that resist the power stroke slow myosin-driven contraction, suggesting that the inherent properties of myosin contribute to the force-velocity relationship in muscle and play an important role in the regulation of cardiac power output. Based on our results and kinetic modeling, we propose that force inhibits the mechanical transition associated with ADP release, leading to slowing of the rate of ADP release, the same kinetic step that limits muscle shortening. These results have important implications for cardiac diseases which affect power output, such as heart failure and cardiomyopathies. This work was supported by the American Heart Association (14SDG18850009 to M.J.G.) and National Institutes of Health (R01GM057247 to E.M.O. and K99HL123623 to M.J.G.).

### 1005-Plat
**Effect of Mutations in cMyBP-C on Sarcomere Mechanical Function**

**Djordje Nedic**[1], Marina Svicevic[1], Boban Stojanovic[1], Michael A. Geeves[2], Thomas Irving[3], Richard J. Gilbert[4], Srboljub M. Mijailovich[4].
[1]Faculty of Science, University of Kragujevac, Kragujevac, Serbia, [2]School of Biosciences, University of Kent, Canterbury, United Kingdom, [3]Illinois Institute of Technology, Chicago, IL, USA, [4]College of Science, Northeastern University, Boston, MA, USA.

A significant number of cardiac myopathies in children and adults are caused by mutations in the cMyBP-C gene. In disease, cardiac output is compromised by altered cardiac muscle fiber contractility due to modulated interactions between cMyBP with actin or with S2. Comparative measurements in WT and knockout mice ($cMyBP\text{-}C^{-/-}$) muscle fibers have showed increased isotonic shortening velocity, power output and rate of force redevelopment in absence cMyBP-C. Thus, change of only a few amino acids in mutant cMyBP-C, especially in regions rich with phosphorylation sites, may cause significant change in dynamics of muscle contractility. Comparison of measured sliding velocities of actin filaments over the regions of myosin filament with and without cMyBP-C in motility assays have provided molecular insight how these structural changes alter the kinetics of the interactions of cMyBP-C with myosin and actin filaments. We used a multi-scale, computational modelling platform, **MUSICO,** (**MU**scle **SI**mulation **CO**de) to assess the effect of cMyBP-C mutations on sarcomere contraction. This platform includes explicit 3-D sarcomere structures, extensible actin and myosin filaments, various models for the acto-myosin cycles, thin filament regulation via a continuous flexible chain (CFC) model and now cMyBP-C using the kinetic parameters for dynamically forming and disrupting connections between cMyBP-C and actin, derived from the motility studies. We compared the model predictions between different mutations and the corresponding mechanical experiments. The predictions from cMyBP-C sarcomeric model showed significant differences between the mutants, and closely followed observations. This results allow the quantitative evaluation of the role of cMyBP-C in the regulation of sarcomere structure and function, the development of a multi-scale myoarchitectural representation of disease phenotype and the creation of a novel diagnostic and prognostic methodology for tracking disease progression in patients.
Supported by: R01s AR048776 and DC 011528

### 1006-Plat
**The A31P Hcm Mutation in cMyBP-C Disrupts the Structure of the C0 Domain But Does Not Cause Haploinsufficiency in a Population of Older Cats Heterozygous for the A31P Allele**

**Sabine J. van Dijk**[1], Kristina L. Bezold[2], Stacy Mazzalupo[1], Mark D. Kittleson[3], Alla S. Kostyukova[4], Samantha P. Harris[1].
[1]University of Arizona, Tucson, AZ, USA, [2]Stanford University, Stanford, CA, USA, [3]University of California, Davis, Davis, CA, USA, [4]Washington State University, Pullman, WA, USA.

Mutations in MYBPC3, the gene encoding the muscle regulatory protein cardiac myosin binding protein-C (cMyBP-C), are among the most common causes of hypertrophic cardiomyopathy (HCM) in both people and cats. However, despite the high prevalence of mutations in MYBPC3, relatively little is understood regarding how mutations lead to disease. One possibility is that some point mutations alter cMyBP-C protein structure leading to enhanced degradation and elimination of the mutant protein. If levels of cMyBP-C protein expression are reduced, then haploinsufficiency (lack of sufficient protein) can trigger disease. Here we tested this idea by analyzing the impact of the A31P mutation, linked to HCM in Maine Coon cats, on 1) the in vitro protein structure of the C0 domain of cMyBP-C, and 2) the total protein expression of cMyBP-C in myocardium of aged cats heterozygous for the A31P mutation. In vitro results demonstrated that the A31P mutation disrupts folding of the C0 domain as shown by three independent methods: altered epitope recognition on Western blots; changes in sensitivity to proteolytic degradation; and reduced β-sheet content assessed by circular dichroism. Western blots of endogenous cMyBP-C obtained from myocardial samples also suggested that C0 structure is altered in vivo because an antibody that preferentially recognizes C0 reacted less with A31P cMyBP-C compared to wild-type cMyBP-C. However, despite these significant structural differences, the A31P cMyBP-C was incorporated into sarcomeres and total cMyBP-C protein (wild-type plus mutant) was similar in wild type and heterozygous A31P cats. These results suggest that despite protein folding abnormalities, the A31P mutation does not lead to haploinsufficiency in the population of older heterozygous cats studied here. Supported by NIH R21HL093603.

**1007-Plat**

**Cell and Myofibril Contractile Properties of hiPSC-Derived Cardiomyocytes from a Patient with a MYH7 Mutation Associated with Familial Cardiomyopathy**

**Josè Manuel Pioner**[1,2], Kai-Chun Yang[3], Lil Pabon[4], Alice Ward Racca[2], Mark Y. Jeong[5], Christian I. Childers[6], Jesse Macadangdang[2], Chiara Tesi[1], Corrado Poggesi[1], Deok-Ho Kim[2], Charles E. Murry[4], Michael Regnier[2].
[1]Experimental and Clinical Medicine, University of Florence, Florence, Italy, [2]Bioengineering, University of Washington, Seattle, WA, USA, [3]Medicine (Div. Cardiology), University of Washington, Seattle, WA, USA, [4]Pathology, University of Washington, Seattle, WA, USA, [5]Medicine (Div. Cardiology), University of Colorado, Denver, CO, USA, [6]University of Washington, Seattle, WA, USA.

Myosin heavy chain 7 (MYH7) mutations are associated with familial cardiomyopathies (FCM) and result in a high rate of sudden cardiac death. Human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) have recently shown promise as a model for studying FCM. We identified a cohort with familial cardiomyopathy (FCM) associated with a MYH7 mutation (E848G) and middle-age onset of systolic dysfunction and arrhythmias. hiPSC-CMs from patient affected (FCM-CMs) and non-affected (WT-CMs) individuals were generated from skin fibroblasts. Here we report, for the first time, contractile properties of isolated myofibrils from these cultured hiPSC-CMs for comparison using cultured cells and 3D engineered cardiac tissue (3D-ECT) constructs. Isolated myofibrils were obtained from differentiation day 20 hiPSC-CMs that were replated onto fibronectin-coated nanopatterned cover slides and matured in culture for an additional 60 days to obtain elongated and aligned myofibrils. This procedure produced hiPSC-CMs that were usually $> 100+$ μm in length. hiPSC- FCM-CMs and WT-CMs were harvested and skinned in a rigor solution containing 1% Triton and contractile properties of single or small bundles of myofibrils were measured in a custom built apparatus with rapid solution switching capabilities. During maximal calcium activation FCM-CM myofibrils produced approximately half the amount of force of WT-CM myofibrils, but preliminary data suggests no differences in the kinetics of force development or relaxation. This compares well with 50 day cardiomyocytes plated on nanopatterned surfaces or seeded into 3D-ECT constructs, where shortening and force (respectively) of FCM-CMs was much less than for WT-CMs, with no difference in calcium transient amplitudes. We speculate this early stage contractile deficit may contribute to disease development and conclude hiPSC-FCM-CMs can be a viable model for mechanical studies of cardiomyopathies in vitro.

**1008-Plat**

**Modulation of Cardiac Twitch Dynamics by the Troponin I Inhibitory Region**

Yasser Aboelkassem[1], Jordan Bonilla[2], **Stuart G. Campbell**[1].
[1]Biomedical Engineering, Yale University, New Haven, CT, USA,
[2]Electrical Engineering, California Institute of Technology, Pasadena, CA, USA.

We have created a computational model of cardiac thin filament regulation that includes a representation of the troponin I inhibitory region (or inhibitory peptide, IP) and its binding interactions with actin. According to a canonical view of thin filament activation, IP-actin binding prevents movement of tropomyosin out of its blocked position under low $Ca^{2+}$ conditions. $Ca^{2+}$ binding to troponin C (TnC) causes dissociation of the IP from actin, and permits tropomyosin transition. Instead of assuming that IP-actin interactions are infinitely strong in the absence of $Ca^{2+}$, our model allows some spontaneous IP-actin dissociation. We have used the energetic cost of $Ca^{2+}$-free dissociation ($\Delta G$) as a free parameter to determine whether the model can recapitulate changes to the IP. For instance, lowering $\Delta G$ while keeping all other model parameters constant increases the $Ca^{2+}$ sensitivity of steady-state force in model simulations. These model results closely resemble experiments in which the IP is mutated (T144P; Tachampa et al., Circ Res 101:1081, 2007). We hypothesize that alterations to the IP in the form of cardiomyopathic mutations or phosphorylation have the ability to tune the dynamic $Ca^{2+}$ sensitivity of cardiac muscle, altering the magnitude and time course of twitches. Twitch simulations demonstrate that lowering $\Delta G$ from infinity to 6.75 kJ/mol increases the magnitude and duration of contraction by 17 and 20%, respectively. These results suggest that twitch dynamics can be modified substantially by the energy of IP-actin binding. They further suggest that the model can be used to explore the effects of IP mutations and posttranslational modifications.

## Platform: Protein Lipid Interactions II

**1009-Plat**

**An Unusual Membrane-Protein Topology for Sensing Bilayer Thickness and Triggering Bacterial Biofilm Formation**

**Sandro Keller**, Jana Broecker, Sebastian Fiedler, Katharina Gimpl, Martin Textor.
Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany.

We propose a new topology of a bacterial membrane protein that serves to sense changes in mechanical bilayer properties and, through interactions with a potassium ion channel, to trigger biofilm formation (1). The membrane-associated protein MstX (2) from Bacillus subtilis self-inserts into lipid bilayers in such a way that its four helices lie parallel to the bilayer plane, with two helices residing in each of the two apposing lipid headgroup regions. This topology suggests a functional role of the protein as a modular entity for sensing membrane properties such as bilayer thickness and hydration, as indicated by a combination of different optical-spectroscopic techniques probing protein structure and dynamics as a function of the effective hydrophobic diameter of the membrane core. Accordingly, increasing membrane thickness or decreasing membrane hydration results in a loosening of the helical-bundle structure of MstX, which, through physical but noncovalent contacts, affects the open probability or the single-channel conductance of YugO, a hitherto uncharacterised potassium ion channel essential for biofilm formation that is encoded in the same bicistronic operon as MstX (3). Leakage of potassium ions through YugO finally initiates known signal transduction cascades that result in the derepression of a set of genes required for biofilm formation.

References:

(1) Broecker et al. J. Am. Chem. Soc. 2014, accepted, DOI 10.1021/ja5064795
(2) Roosild et al. Science 2005, 307, 1317
(3) Lundberg et al. PLoS One 2013, 8, e60993

**1010-Plat**

**Insights into the Specificity of Neisserial Opa Protein Interactions with Human Receptors**

**Jennifer N. Martin**[1], Ryan H. Lo[1], Alison K. Criss[2], Linda Columbus[1].
[1]Chemistry, University of Virginia, Charlottesville, VA, USA,
[2]Microbiology, Immunology and Cancer Biology, University of Virginia School of Medicine, Charlottesville, VA, USA.

Pathogenic *Neisseria gonorrhoeae*, the causative agent of gonorrhea, possess a family of outer membrane proteins referred to as opacity-associated (Opa) proteins. These Opa proteins are β-barrel outer membrane proteins that bind to human host cell receptors, inducing engulfment of the bacterium. To date, there have been over 300 distinct *opa* alleles sequenced. The differences in sequence have stemmed primarily from recombination events, and are most pronounced in two regions of the protein in the second and third extracellular loops (termed hypervariable regions, HV1 and HV2). These HV regions are responsible for determining receptor specificity. The most abundant Opa family engages human CEACAM receptors (carcino-embryonic antigen-like cellular adhesion molecules). While the Opa protein family has conserved structural elements, the molecular determinants of the receptor interactions are unknown. We