regulatory units. Thus the apparent cooperativity of activation is an emergent property caused by the ensemble of cTnC populating distinct affinity-states that arise from the coordinated super architecture of the sarcomeric lattice; due to the nature of the cTnC-Ca$^{++}$ binding event, cTnC is biased toward binding Ca$^{++}$ in affinity-states with higher Ca$^{++}$-affinity as activation progresses.

1809-Pos Board B579
Incorporating Cooperativity into Huxley-Type Cross-Bridge Models in Thermodynamically Consistent Way
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We present a mathematical model of actomyosin interaction, as a further development of a cross-bridge model that links mechanical contraction with energetics [Yen et al., 1999, Biomed. Eng. 28: 629, 2000]. The former model is composed of the Huxley-type model for cross-bridge interaction and the phenomenological model of calcium - induced activation. The purpose of the new model was to replace the phenomenological description. To introduce mechanistic description of the activation, cooperativity effects should be taken into account.

The aim of this work is to incorporate cooperativity into Huxley-type cross-bridge model in thermodynamically consistent way.

While the Huxley-type models assume that cross-bridges act independently from each other. Here we take into account that each cross-bridge is influenced by its neighbors. We assume that the muscle contraction can be described by ensemble of cross-bridge groups. For simplicity, the groups consist of five consecutive cross-bridges, out of which the first and the last ones are always in unbound state as boundary conditions. Cooperativity is introduced by taking into account that binding of calcium or cross-bridge leads to displacement of tropomyosin. Since tropomyosin connects all cross-bridges in a group, the elastic deformation of tropomyosin will influence free energy of the group as well as reaction kinetics.

The model parameters were found by optimization from the linear relation between oxygen consumption and stress-strain area [Husano et al., Circ. Res.: 61: 318, 1987] as well as experimentally measured stress dynamics of rat trabecula [Janse et al., Am. J. Physiol.: 269: H676, 1995]. We have found a good agreement between the optimized model solution and experimental data. In addition, model solutions demonstrate the cooperative effects.

1810-Pos Board B580
From Single Molecule Fluctuation to Muscle Contraction: A Brownian Model of A.F. Huxley’s Hypotheses
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Force generation during muscle contraction is the result of thermally fluctuating cyclical interactions between myosin and actin, which together form the actomyosin complex. Normally, these fluctuations are modelled using transition rate functions that are based on muscle fibre behaviour. However, this reduces the predictive power of such models. Therefore, we propose an alternative approach that incorporates diffusion and uses the direct observations of actomyosin dynamics reported in the literature. We precisely estimate the actomyosin potential bias to obtain a Brownian ratchet model that reproduces the complete cross-bridge cycle.

The model is validated by simulating several macroscopic experimental conditions, while its interpretation is compatible with two different force-generating scenarios.

1811-Pos Board B581
Reverse Computational Modeling: From Muscle Mechanics to the Function of Sarcomeric Proteins
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Spatially-explicit mathematical models of muscle sarcomeres (e.g. Daniel et al., 1998, Smith et al., 2008) incorporate information about the position of each molecule in the myofilament lattice and are useful because they can reproduce geometrical effects due to filament architecture and the variable alignment of actin binding sites and myosin heads. Their main disadvantage is that they have many more parameters (for example, filament stiffness values, Ca$^{++}$ binding affinities, etc.) than conventional Huxley-type models. As a result, most spatially-explicit models to date have been used to make ‘forward predictions’. That is, investigators have assigned a plausible value to each model parameter and then run simulations to predict contractile function (tension-pCa curves, measurements of tension recovery, etc.) under various conditions. This can determine the impact on muscle function of altering the biophysical properties of a selected sarcomeric protein. We have developed our own spatially-explicit model (FiberSim) and are currently using it in the reverse direction. Our aim is to predict the functional behavior of many sarcomeric proteins by adjusting the values of the parameters that describe their biophysical behaviors until the resulting simulations match real experimental data records. We have recently succeeded in reproducing tension-recovery (ktr) records obtained at different levels of Ca$^{++}$ activation using permeabilized myocardial samples from diabetic rats. These samples only contain the slow β isoform of myosin heavy chain and the simulations thus predict a complete kinetic scheme for this isoform’s interaction with actin. We are currently extending our work to predict the fast α isoform’s kinetic scheme as well by optimizing the fit between the simulations and ktr records obtained from control myocardial samples which contain 30% α and 70% β myosin.

1812-Pos Board B582
Cardiomyopathy-Related Mutations (E244D, K247R, D270N, and K273E) in the H2-Helix of Cardiac Troponin T Have Varied Effects on Myofila-
mament Responsiveness to Calcium and Crossbridge Recruitment Dynamics
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Two hypertrophic cardiomyopathy (HCM)-related mutations, E244D and K247R, and two dilated cardiomyopathy (DCM)-related mutations, D270N and K273E, have been identified along a centralized helix of cardiac troponin T (cTnT). This helix, termed H2(T), is centrally located in the core domain of cardiac troponin, interacting with cardiac troponin I and troponin C. This indicates a functional role of H2(T) in translating conformational changes sensed in troponin C and troponin I to the rest of the thin filament, but this structure-function relationship is not well understood. To determine the significance that disease-related alterations in the structure of H2(T) have in altering contractile function, we measured the contractile properties of rat cardiac muscle fibers containing rat cTnT variants cTnT(E244D), cTnT(K247R), cTnT(D270N), or cTnT(K273E) corresponding to human E244D, K247R, D270N, or K273E mutations, respectively. We measured simultaneous force production and ATPase activity, as well as force responses (F(t)) to step-length perturbations in demembranated cardiac muscle fibers activated at various [Ca$^{++}$], at both sarcomere lengths 2.0 and 2.3 μm. Fibers containing cTnT(E244D) exhibited an increase in myofilament Ca$^{++}$ sensitivity when compared to those containing wild-type (WT)-cTnT. In addition, crossbridge recruitment dynamics, as estimated by model-predictions of F(t) and ktr measurements, were slower in fibers containing cTnT(E244D) and cTnT(K273E), which was also seen in fibers containing cTnT(D270N). Ca$^{++}$ sensitivity of fibers containing cTnT(K247R) or cTnT(D270N) was less than that of fibers containing WT-cTnT. Furthermore, maximal ATPase activity was slightly but significantly increased in fibers containing cTnT(E244D) or cTnT(K273E). These findings suggest that mutations along H2(T) influence the cTnT-modulated mechanisms of myofilament activation and crossbridge recruitment dynamics, and may contribute in part to cardiac dysfunction associated with HCM and DCM diseases.

1813-Pos Board B583
Changes in the Myocardial Expression of Tropomyosin Isoforms Modulate Troponin T-Mediated Cardiac Thin Filament Activation
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The spatial distribution of the troponin complex (Tn) on the thin filament, and as a result, the functional role of Tn in cardiac thin filament activation depends on structural interactions between the N-terminus of troponin T (T1) and the overlapping ends of tropomyosin (Tm). Thus, T1-Tm interactions have an influential role in regulating cardiac thin filament activation. Structural alterations in T1 have been shown to diminish cardiac activation and in view of the physical interactions between T1 and Tm, it is conceivable that the effect of T1 on cardiac activation can also be modulated by structural alterations in Tm. Therefore, there is a need to understand how changes in Tm influence T1-mediation of cardiac activation. To better understand how structural changes in Tm influence T1-mediated cardiac activation, we studied contractile function by reconstituting mouse cardiac troponin T (McTnT) deletion proteins, McTnT I-44 deletion and McTnT 45-74 deletion, onto detergent-skinned papillary fibers isolated from hearts of transgenic mice expressing McTnT I-44 deletion and McTnT 45-74 deletion, onto detergent-skinned papillary fibers isolated from hearts of wild-type mice containing α-Tm. Our preliminary results show that the T1 deletions induced significant functional alterations in wild-type fibers. Interestingly, the T1-deletion-induced alteration of cardiac function was further modulated by the myocardial expression of β-Tm. For example, the McTnT I-44 deletion-induced reduction
in myofilament Ca\(^{2+}\) sensitivity was abolished by the expression of β-Tm. Furthermore, the MctnT 45-74 deletion-induced reduction in cooperativity of force production was more pronounced under a β-Tm background. Thus, our data shows that changes in the isoform expression of Tm modify T1-dependent cardiac function, indicating that T1-Tm interactions exert a modulatory role in regulating cardiac thin filament activation.

1814-Pos Board B584
Cardiac Troponin I Phosphorylation at ser149 by Protein Kinase A: A Potential Modulator of Myocardial Contractility
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In the heart, stimulation of β-adrenergic pathway and subsequent activation of protein kinase A (PKA) is known to increase myocardial contractility. The increase in contractility is, in part, due to target phosphorylation of troponin I (Tnl). In this study, we sought to identify novel target sites for PKA that could potentially contribute to this increase in contractility. To induce phosphorylation of Tnl, cardiac and fast skeletal muscle from 3-4 month old Sprague Dawley rats was mechanically disrupted and demembranated followed by incubation with the catalytic subunit of PKA (50U PKA/3mg tissue, 0-30 min). To identify target specific phosphorylation on fast skeletal (fsTnl) or cardiac (cTnl) Tnl, western blot analysis with phospho-specific antibodies was performed. PKA treatment increased phosphorylation of cTnl at ser22/23, as expected, but also at ser149. Similarly, PKA treatment increased phosphorylation of fsTnl at ser117, which is the equivalent to ser149 in cTnl. Accordingly, fsTnl demonstrated no observable phosphorylation at ser22/23. Adenosine monophosphate activated kinase (AMPK) has been shown to target ser149 of cTnl. Therefore, to validate PKA-dependent phosphorylation of cTnl at ser149, hearts were excised and perfused with AICAR, a known activator of AMPK. AICAR-perfused hearts demonstrated a time-dependent increase in phosphorylation of cTnl at ser149. These results demonstrate that PKA-dependent phosphorylation can target ser149 in cTnl and, equivalently, ser117 in fsTnl. The functional consequence of this target site phosphorylation and how it impacts contractility is currently under investigation.

1815-Pos Board B585
D230N Mutation in Tropomyosin and R92L Mutation in Cardiac Troponin C have Strikingly Different Impact on Calcium-Regulated Activation of Cardiac Myofilaments
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1Washington State University, Pullman, WA, USA, 2Albert Einstein College of Medicine, Bronx, NY, USA, 3Dilated Cardiomyopathy (DCM) and Familial Hypertrophic Cardiomyopathy (FHC) are pathological heart conditions mainly associated with sarcomeric mutations. Defective binding and Ca\(^{2+}\) sensitivity in DCM and FHC may cause by altered Ca\(^{2+}\) handling and Ca\(^{2+}\) sensitivity in DCM and FHC. The mechanism of doxorubicin-induced suppression of cardiac ankyrin repeat protein (CARP, ANKRD1) is a transcriptional regulatory protein that is extremely susceptible to doxorubicin, however, the mechanism(s) of doxorubicin-induced suppression and its specific role in cardiomyocyte biology remains to be elucidated. In this study, we report that treatment of cardiomyocytes with doxorubicin resulted in complete suppression of CARP promoter activity, decreased CARP protein levels, and marked sarcomere disarray. Transfection of CARP siRNA in cardiomyocytes resulted in a complete depletion of CARP and significant disruption of sarcomere ultrastructure. Adenoviral overexpression of CARP, however, was unable to rescue the doxorubicin-induced sarcomere disarray phenotype. GATA4 has previously been shown to regulate CARP, thus we examined the role of GATA4 in doxorubicin-induced CARP dephosphorylation. Cardiomyocytes treated with doxorubicin showed concomitant depletion of CARP and GATA4 protein levels. GATA4 siRNA inhibitors while GATA4 overexpression enhances CARP promoter activity in cardiomyocytes. Both GATA4 and CARP siRNA significantly repressed titin and actin promoter activity. These data show that in cardiomyocytes transcription factor GATA4 is upstream of CARP and that doxorubicin induces a rapid down-regulation of GATA4 resulting in inhibition of CARP transcription. Our data further support a role for a GATA4/CARP signaling axis in sarcomere maintenance and that suppression of this pathway contributes, in part, to the overall pathophysiology of doxorubicin cardiomyopathy.

1818-Pos Board B588
MLCK moves on Actin Filament and Stress Fiber of Smooth Muscle Cells Feng Hong1, Ruby Sukhrari, Michael Carter1, Mariam Ba1, Michael P. Walsh2, Josh E. Baker1, Christine P. Cremo1. 1University of Nevada School of Medicine, Reno, NV, USA, 2University of Calgary, Calgary, AB, Canada.
We are interested in the mechanism of phosphorylation of smooth muscle myosin (SMM) by the myosin light chain kinase -calmodulin-Ca\(^{2+}\) complex (MLCK-CaM-Ca\(^{2+}\)). This reaction is required for activation of SMM catalytic activity and smooth muscle contraction. In previous studies we characterized tightly-bound SMM-MLCK-CaM complexes in an in vitro model system and demonstrated that SMM-MLCK-CaM complexes co-purified from smooth muscle. Moreover, using total internal reflectance fluorescence microscopy (TIRF), we observed dynamic interactions between single MLCK and SMM molecules by visualizing quantum dot-labeled MLCK (QD-MLCK) interacting with SMM aligned on actin filaments. We have also observed QDs-MLCK moving on Actin Filament and Stress Fiber of Smooth Muscle Cells.