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^{2+} binding event, cTnC is biased toward binding Ca^{2+} in affinity-states with higher Ca^{2+}-affinity as activation progresses.

1809-Pos Board B579

Incorporating Cooperativity into Huxley-Type Cross-Bridge Models in Thermodynamically Consistent Way

Mari Kalda, Pearu Peterson, Marko Vendelin.
Laboratory of Systems Biology, Institute of Cybernetics, Tallinn University of Technology, Tallinn, Estonia.

We present a mathematical model of actomyosin interaction, as a further development of a cross-bridge model that links mechanical contraction with energetics [Vendelin et al., Ann. 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 consequative 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 [Hisano et al., Circ. Res.: 61: 318, 1987] as well as experimentally measured stress dynamics of rat trabecula [Jansse 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 cooperativity effects.

1810-Pos Board B580

From Single Molecule Fluctuation to Muscle Contraction: A Brownian Model of A.F. Huxley's Hypotheses

Lorenzo Marcucci, Toshio Yanagida.
Osaka University, Suita (Osaka), Japan.

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 fiber 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

Kenneth S. Campbell[1,2], Qiang Ye[2,3].
[1]Department of Physiology, University of Kentucky, Lexington, KY, USA, [2]Center for Muscle Biology, University of Kentucky, Lexington, KY, USA, [3]Department of Mathematics, University of Kentucky, Lexington, KY, USA.

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^{2+} 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 alternative '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 (k_{tr}) records obtained at different levels of Ca^{2+} 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 interactions 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 k_{tr} 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 Myofilament Responsiveness to Calcium and Crossbridge Recruitment Dynamics

Steven J. Ford, Srilakshmi Mallampalli, Murali Chandra.
Washington State University, Pullman, WA, USA.

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 $cTnT_{E245D}$, $cTnT_{K248R}$, $cTnT_{D271N}$, or $cTnT_{K274E}$ 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^{2+}], at both sarcomere lengths 2.0 and 2.3 μm. Fibers containing $cTnT_{K274E}$ exhibited an increase in myofilament Ca^{2+} sensitivity when compared to those containing wild-type (WT)-cTnT. In addition, crossbridge recruitment dynamics, as estimated by model-predictions of $F(t)$ and k_{tr} measurements, were slower in fibers containing $cTnT_{K274E}$, a trend that was also seen in fibers containing $cTnT_{D271N}$. Ca^{2+} sensitivity of fibers containing $cTnT_{K248R}$ or $cTnT_{D271N}$ was less than that of fibers containing WT-cTnT. Furthermore, maximal ATPase activity was slightly but significantly increased in fibers containing $cTnT_{E245D}$ or $cTnT_{K248R}$. 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

Ranganath Mamidi, Srilakshmi Mallampalli, Murali Chandra.
Washington State University, Pullman, WA, USA.

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. However, there is a lack of understanding of how structural 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 1-44 deletion and McTnT 45-74 deletion, onto detergent-skinned papillary fibers isolated from hearts of transgenic mice expressing β-Tm. Control experiments were performed by reconstituting the McTnT 1-44 deletion and McTnT 45-74 deletion proteins 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 1-44 deletion-induced reduction