757-Pos
The Role of Surface Physics in Motility
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Nearly all research in motility has focused sole attention to the solid active elements in the cell while regarding the fluid components (the cytoplasmic and myoplasmic fluids) as primarily passive elements in force generation and movement. The release of the products of hydrolysis has a major effect on the surface energy in the fluid boundary making the fluid proteophobic directly producing force and movement of the structure it is interfaced with.
Considering the fluid surface physics elucidates the following:
1. In muscle a change in surface tension at the fluid-filament boundary of only 6 dynes/cm will producing an increase in proteophobicity resulting in a contractile force equal to the maximum that striated muscle can produce.
2. The optimum position for hydrolysis of ATP to most effectively produce the force on a cargo attached to a microtubule at the 12 o'clock position will be shown to be at the 5 or 7 o'clock position.
3. The viral packing motor function is explained by the release of phosphate ions in the hydrolysis of ATP around the DNA outside the capsid. The free surface energy at the fluid-DNA boundary becomes elevated by the ions which forces the DNA inside where the DNA-DNA interface (as it folds) is less.

758-Pos
Mathematical Model of Multiple Myosin System with Measurement Probes, Focusing on Energy Efficiency
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Myosin is a molecular motor, which slides along actin filaments during ATP hydrolysis. Experimental results of single and multiple molecule measurements show that myosin can slide 15 - 200 nm, which is much lager than molecular size of myosin. To understand sliding mechanism of myosin system, we have developed the model of multiple myosin system with modifying potential function, and analyzed structure of the model. Our results suggest that energy consumption per unit step would be lower by connecting myosins, which is consistent with larger interaction length (IL) experimentally estimated previously (60 - 200 nm, with surface assay). In this study, we will show characteristic structure of potential function of the model to satisfy IL of single and multiple myosin system.
Additionally, in order to verify the model with experimental data, here, we construct model including characteristics of measurement probes (ex. scanning probe (SP), optical tweezers (OT)), and simulate movement of myosin system attached to measurement probes (SP or OT). In order to take into account effects of measurement probes, we construct model of myosin system attached to probes via spring, and simulate movement of myosin system along periodic potential with Langevin equation method. We test effects of spring constant and size of probes. As a result, sliding velocity with SP becomes slower than that with OT, then displacement generating process clearly observed with SP. We will show and discuss our model and experimental data at the meeting.

759-Pos
Actomyosin ADP-States, Non-Hyperbolic Force-Velocity Relation and Processivity of Myosin II in Fast Skeletal Muscle
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The force-velocity relation of striated muscle is incompletely understood with respect to the molecular basis for the maximum shortening velocity and for the non-hyperbolic shape at high forces (low velocities). These, and related issues, are here elucidated using a four-state actomyosin cross-bridge model. Exploration of the parameter space of the model suggests that an actomyosin-ADP state (AM*ADP) with a closed nucleotide pocket, or rather the strain-dependent transition out of this state, has a pivotal role in influencing both the maximum shortening velocity and the shape of the force-velocity relation in the high-force region. Another model property that influences the shape of the high-force region is the detailed dependence of cross-bridge attachment rate on cross-bridge strain. Here, the modelling results argue against ideas of high attachment rate for highly strained cross-bridges. Finally, evidence is presented that actin attached myosin heads (in the AM*ADP state) have the appropriate structural and kinetic properties to position the partner head for rapid attachment to the next site along the actin filament. This would be reminiscent of the role of the corresponding state of myosin V and could form the basis for limited processivity of muscle myosin II to increase the power output during shortening against intermediate loads.

760-Pos
Interactions Between Connected Half-Sarcomeres Produce Emergent Mechanical Behavior in a Mathematical Model of Muscle
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Most reductionist theories of muscle attribute a fiber's mechanical properties to the scaled behavior of a single half-sarcomere. Mathematical models of this type can explain many of the known mechanical properties of muscle but have to incorporate a passive mechanical component that becomes ~300% stiffer in activating conditions to reproduce the force response elicited by stretching a fast mammalian muscle fiber. The available experimental data suggests that titin filaments, which are the mostly likely source of the passive component, become at most ~30% stiffer in saturating Ca2+ solutions. The work described in this manuscript used computer modeling to test an alternative systems theory that attributes the stretch response of a mammalian fiber to the composite behavior of a collection of half-sarcomeres. The principal finding was that the stretch response of a chemically permeabilized rabbit psoas fiber could be reproduced with a framework consisting of 300 half-sarcomeres arranged in 6 parallel myofibrils without requiring titin filaments to stiffen in activating solutions. Ablation of inter-myofibrillar links in the computer simulations lowered isometric force values and lowered energy absorption during a stretch. This computed behavior mimics effects previously observed in experiments using muscles from desmin-deficient mice in which the connections between Z-disks in adjacent myofibrils are presumably compromised. The current simulations suggest that muscle fibers exhibit emergent properties that reflect interactions between half-sarcomeres and are not properties of a single half-sarcomere in isolation. It is therefore likely that full quantitative understanding of a fiber's mechanical properties requires detailed analysis of a complete fiber system and cannot be achieved by focusing solely on the properties of a single half-sarcomere.

761-Pos
Micro-Mechanical Model of Muscle Contraction
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A new mathematical model of skeletal muscle contraction based on two recent single myosin molecule experiments is proposed. First, a single head of Myosin II attached to a large microneedle in the presence of an actin filament shows several 5.5 nm steps in one preferred direction per ATP cycle [1]. Second, a single head of Myosin VI is attached to a bead, trapped in a laser, in the presence of an actin filament and rapid (76 microseconds) and large (250 nm) displacements are imposed to the bead, revealing that the probability for the actin-myosin complex to switch from a weakly attached state to a strongly attached state increases when the scan is performed in the opposite direction of the natural movement of the head itself [2]. We refer to this effect as Strain Sensor (SS). The behavior of the muscle, at the fiber length scale, is interpreted on these new experimental evidences, at the molecular motor scale, allowing the definition of a micro-mechanical model of the contraction. The stepping behavior is modeled with a diffusive process in a well defined potential, following the theory of the Brownian ratchets, while the SS affects the jump process between the attached and the detached state of the myosin head. The model is able to reproduce globally the behavior of the muscle, in its short time scale, related to the power stroke, and in its long time scale, related to the actin-myosin cycle. The response of the model is analyzed by a stochastic simulation of the Langevin equations associated to a population of parallel distributed myosin heads.
[1] Kitamura, Tokunaga, Esaki, Iwane, Yanagida. Biophysics (2005)
[2] Iwaki, Iwane, Shimokawa, Cooke, Yanagida. Nat. Chem. Biol. (2009)

Muscle Regulation I

762-Pos
Comparison of the Binding of the Switch Regions of Cardiac Troponin-I and Skeletal Troponin-I to the Functional N-Domain of Human Cardiac Troponin-C
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In the heart, there are two isoforms of troponin-I (TnI) that are developmentally regulated. The slow skeletal TnI (ssTnI) is the sole isoform expressed in the embryonic or neonatal heart, while cardiac TnI (cTnI) is expressed exclusively in the adult heart. One important distinction between ssTnI and cTnI relates to differences in myofilament Ca^{2+}-sensitivity of force development under acidic pH conditions. Hearts expressing ssTnI show heightened Ca^{2+}-sensitivity compared with hearts expressing cTnI under basal conditions. This isoform-specific

difference is greatly accentuated during acidosis, which is often associated with ischemia of myocardial infarction. *In vitro* and *in vivo* functional studies have shown that replacement of cTnI with ssTnI results in a marked enhancement of myofilament Ca^{2+}-sensitivity at acidic pH conditions. Recent reports have indicated that this effect can be ascribed to amino acid sequence differences in ssTnI and cTnI and in particular to a critical A162H substitution in the switch region. In this study, we have used NMR spectroscopy to examine the binding of the switch regions of ssTnI ($sTnI_{115-131}$) and cTnI ($cTnI_{147-163}$) to the N-domain of cardiac troponin-C (cNTnC) at physiological and acidic pH conditions. The results show that the affinity of $sTnI_{115-131}$ for cNTnC•Ca^{2+} (K_D ~50uM) is ~3-fold stronger than that of $cTnI_{147-163}$ (K_D ~150uM), but neither are affected by a pH change from 7 to 6. The pKa of H130 in $sTnI_{115-131}$ is 6.2 when free and 6.7 when bound to cNTnC•Ca^{2+}. We have also used {^{1}H, ^{15}N}-HSQC NMR spectroscopy to monitor the pKa changes of cNTnC•Ca^{2+} from peptide free to peptide bound states. The implications of these results will be discussed in the context of structure and function of myofilament protein interactions.

763-Pos

Structure and Dynamics of Cardiac Troponin C using Paramagnetic Relaxation Enhancement Derived Distances

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We have prepared four spin-labeled single cysteine mutants of isolated cardiac TnC (cTnC) in order to examine the conformation of the Ca^{2+}-loaded *N*-domain and the interdomain dynamics using PRE-NMR. The long-range PRE distances (10 - 30 Å) measured within the regulatory *N*-domain were compared to TnC structures in the Protein Data Bank. Q-factor statistics were used to rank all available TnC PDB structures according to their agreement with our experimentally derived distances with scores ranging from 0.16 (best) to 0.30 (worst). The energy minimized solution structure of isolated human cTnC (1AJ4, Sia *et al*, JBC 1997) demonstrated the best correlation with our PRE data for the *N*-domain. Interdomain dynamics of our isolated cTnC were also examined by comparing PRE distances to available ensembles of TnC structures. Our results indicate that isolated cTnC is more compact than the skeletal TnC isoform, and that the central domain linker is highly flexible with a defined range of relative domain orientations. We also present a simple approach for modeling of the spin label position and mobility using PRE distances which is applicable to all spin-labeled systems for which there are existing structural models.

764-Pos

Protein Kinase a Phosphorylation of Cardiac Troponin I Prevents Cardiac Hypertrophy in Mice

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R21C is the only FHC-associated mutation located in the N-terminal domain of cardiac TnI (cTnI) and is within the consensus sequence for PKA phosphorylation (RR_{21}RSS). We have developed an R21C cTnI knock-in (KI) mouse model and have evaluated the mouse hearts for biochemical, biophysical, structural and functional changes. Our results show that the R21C KI mice (heterozygous;R21C+/- and homozygous;R21C+/+) developed the FHC phenotype as evidenced by the presence of hypertrophy and fibrosis. Some hypertrophic markers such as, ANP, BNP and β-MHC were found elevated at a late age (18 months). The R21C+/- and R21C+/+ mice had decreased phosphorylation at Ser23/24 (~18% and 90%, respectively) compared to WT mice. Top down mass spectrometry of cTnI from the R21C+/- mice demonstrated a molar ratio of 1:4 R21C:WT in the hearts. Using three different methods to sacrifice the WT and mutant mice, we did not find any significant decrease in the Ca^{2+} sensitivity of force upon PKA treatment. Western blot analysis of these mice showed that the endogenous cTnI in the WT and R21C+/- mice is completely phosphorylated at Ser23/24. However, when mice were treated with propanolol (β-adrenergic receptor antagonist) before sacrifice, the Ca^{2+} sensitivity was decreased after PKA treatment of the WT (0.25 pCa) and R21C+/- mice (0.14 pCa). In contrast the R21C+/+ mice did not show a significant decrease in Ca^{2+} sensitivity after PKA treatment. No significant changes were found in the maximal force in all three mice, before and after PKA treatment. Our results suggest that the primary mechanism for producing hypertrophy in the R21C mice results from the impaired ability of the myofilament to respond to the desensitizing effects of PKA phosphorylation. Supported by NIH grant HL042325

765-Pos

Structure and Dynamics of the Mobile Domain of Troponin I by SDSL-EPR

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The Troponin (Tn) molecular switch contains highly dynamic regions which allow for Ca^{2+} induced conformational changes to be propagated through to the thin filament. The Mobile domain (Md) of TnI, a secondary thin filament binding domain, is a key player in this process. The functional importance of the Md is also highlighted through the clustering of cardiomyopathy mutations. Structural elucidation of this region by traditional methods is often limited by the absence of key thin filament binding partners. Current Md models describe a highly dynamic region with either a nascent or a well-defined structure. We have utilized Site-Directed Spin Labeling Electron Paramagnetic Resonance (SDSL-EPR) to elucidate the structure of the Md upon interaction with the thin filament. EPR mobility measurements from cysteine scanning of the Md (res. 175-206) in the reconstituted thin filament show a highly dynamic domain in the $+Ca^{2+}$ (ON) state. A decrease in the mobility occurs in the $-Ca^{2+}$ (OFF) state, indicating interaction with the thin filament. Further, trends in the mobility of the EPR label reveal two helical structural components within the Md (res. 175-179 & 192-202). Conventional EPR methods were used to measure three interspin distances (176/178, 176/179 & 176/180) which further confirm this assignment. Double Electron-Electron Resonance (DEER) was used to measure the longer interspin distance (178/206) and found that the Md exists in an extended conformation (34 ± 26Å). An extended helical structural model for the interaction of the Md with the thin filament through electrostatic bonding is proposed. Residues involved in cardiomyopathy are found clustered at the interacting interface.

766-Pos

Desensitizing Effect of N-terminal Truncated cTnI In RCM Myofibrils

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Cardiac TnI (cTnI) mutations have been associated with the development of restrictive cardiomyopathy (RCM) characterized by a Ca^{2+} hypersensitivity and diastolic dysfunction in cardiac myofibrils. Whereas cTnI N-terminal deletion (cTnI-ND) caused by restricted proteolysis in cardiac adaptation to stress manifests a lower left ventricular end diastolic pressure and an enhanced ventricular diastolic function. By crossing the RCM cTnI R193H transgenic mice ($cTnI^{193His}$) with cTnI-ND transgenic mice (cTnI-ND) that contain 100% cTnI-ND in the heart, we have obtained double TG mice containing both the cTnI R193H mutant and cTnI-ND. In this study, by using these TG mouse lines, we have investigated the desensitizing effect of cTnI-ND on the RCM cTnI mutant mice and myofibrils. Our survival data for these mice indicated that cTnI-ND greatly reduced the mortality of the RCM $cTnI^{193His}$ mice. Ca^{2+} sensitivity measured in skinned myofibrils confirmed that increased myofibril Ca^{2+} sensitivity was the major mechanism that resulted in impaired relaxation and diastolic dysfunction in RCM $cTnI^{193His}$ mice and that cTnI-ND could reverse the cellular dysfunction by desensitizing the myofibrils to Ca^{2+}. The PKA stimulation assays showed that $cTnI^{193His}$ myofibrils were able to respond to PKA activation, resulting in a right-shift of pCa curve after PKA treatment. However, since the myofibrils from cTnI-ND hearts lacked Ser residues 23 and 24, they had no response to PKA stimulation, showing a similar pCa curve before or after PKA. Our data have, for the first time, demonstrated a desensitizing effect by an endogenous myofibril protein proteolysis without the intervention of β-adrenergic stimulation mediated cTnI phosphorylation. The desensitizing function in cTnI-ND hearts indicates that the removal of cTnI N-terminal extension by restricted proteolysis represents a novel mechanism to improve myofibril relaxation and cardiac diastolic function in cardiac adaptation to hemodynamic and inotropic stresses.

767-Pos

Effects of Pseudo-Phosphorylation of cTnI by P^{21} Activated Kinase-3 (PAK3) on Structure and Kinetics of Ca^{2+}-Induced Cardiac Thin Filament Regulation

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Residue Ser151 of rat cardiac troponin I (cTnI) can be phosphorylated by P21-activated kinase 3 (PAK3). It has been found that PAK3 phosphorylation of cTnI induces an increase in Ca^{2+} sensitivity of myofilament, but detailed mechanism is unknown. We investigated the structural and kinetic effects of phosphorylation of cTnI PAK3 site Ser151 on the Ca^{2+}-induced thin filament regulation. Using