techniques for the genetic manipulation of this model are well established. Our major goal is to establish an isolation method and further refine it to examine the structure and functioning of both native zebrafish cardiac myosin thick filaments and filaments with mutations in the thick filament associated proteins. In the previous isolation techniques for mammalian cardiac thick filaments, the use of potentially damaging proteolytic enzymes such as elastase and calpain has normally been required. We have successfully isolated thick filaments from zebrafish cardiac muscle, using a procedure similar to those for mammalian heart, only this time without the use of any proteolytic enzyme, and have analyzed their structure by negative staining, transmission electron microscopy and SDS-electrophoresis gels. These results could help to improve the 3D reconstruction of the zebrafish cardiac thick filament for the study of the changes in the cardiac thick filament associated with disease processes. Supported by an NIH grant SC1HL096017 to RWK.

810-Pos Board B565

Three-Dimensional Considerations for X-Ray Diffraction Signals that Occur Ahead of Stretch Activation in Insect Flight Muscle

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Stretch activation (SA, delayed rise of force after a stretch) is essential for the asynchronous action of insect flight muscle (IFM). We have shown that a specific reflection spot in the X-ray diffraction pattern (the 111 spot) is the first one to respond to stretch of activated skinned IFM fibers (2011 annual meeting), and the same signal is observed even more prominently in live bumblebees during wing-beat (Iwamoto and Yagi, 2013). Because of its timing to occur and its calcium dependence, we have argued that the signal may reflect a structural change that triggers SA. We have shown that, by using simple model calculations, that the rise of the 111 signal immediately after stretch, accompanied by a reciprocal decrease of the 201 signal, can be explained by a twisting motion of myosin heads already attached to actin filaments. Here, we have extended the initial model and have developed a more comprehensive 3-dimensional model of the myofilament lattice of IFM, in which arrangements of both troponin and myosin heads are taken into consideration. By using this model, the effects of myosin head twisting within a 3-dimensional space of the unit cell are assessed as well as those of troponin movements. It is shown that the movement of myosin heads affects the intensity of the meridional reflection at d = 14.5 nm as has actually been observed in live insects and skinned IFM fibers.

811-Pos Board B566

Modeling Poly-Lysine Interactions with Mutant and Wild Type Cardiac Myosin Subfragment-2

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Familial hypertrophic cardiomyopathy-causing mutations in β-cardiac myosin subfragment-2 destabilize the coiled coil structure which might be related to their phenotypes. Using a synthetic peptide model of this coiled coil region, it was found that the stability of the myosin coiled coil was inversely correlated with the phenotypic severity of the disease-causing mutations. Stability of the coiled coil was measured by luminescence resonance energy transfer in which some of the mutant and wildtype peptides were labeled with donor probes and others were labeled with acceptor probes so the fraction of peptides in a coiled coil could be determined. Similar results were obtained with dynamics simulations of the mutant and wildtype peptides. It was further found that the instability of the coiled coil caused by the mutations could be partially reversed by the addition of poly-D-lysine. Both molecular mechanics and dynamics simulations of the poly-lysine binding to the coiled coil peptide indicated that the poly-lysine of an appropriate length could wrap around the coiled coil by interacting with negatively-charged glutamate side-chains. Further modifications of the sequence of the poly-lysine were found to improve the interaction energy between the complex which may result in increased stabilization of the myosin coiled. These results suggest that it may be possible to design peptides that can counteract the destabilizing effects of specific disease-causing mutations. (Supported by NSF 082736 ARRA.)

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Small Heat Shock Proteins Prevent Titin Aggregation-Induced Stiffening in Human Myocytes

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During stretch of myocytes, mechanically active domains of the giant elastic titin molecule become unfolded. Unfolded proteins typically expose previously buried hydrophobic sites and are under risk to aggregate and lose their function. As for titin, it is unclear whether domain unfolding leads to aggregation and functional impairment. Here we consider that small heat shock proteins (sHSPs), which are abundantly expressed in myocytes, could help prevent titin aggregation. Various sHSPs, including HSP27 and αB-crystallin, are known to translocate under stress conditions preferentially to the sarcomeres, especially the elastic I-band region, where the titin springs are located. We find that HSP27 and αB-crystallin share binding sites on the titin springs, associating with immunoglobulin-like domain (Ig) containing regions, but not with the intrinsically disordered PEVK-domain. In sarcomeres, sHSP-binding to titin occurs independent of the presence of actin filaments and is enhanced by stretch. The titin spring elements behave *in vitro* mainly as monomers. However, unfolded Ig regions aggregate *in vitro*, preferentially under acidic conditions, whereas αB-crystallin protects against aggregation. Disordered titin spring regions do not aggregate. Single human cardiomyocytes in which titin Ig unfolding is promoted reveal elevated stiffness under acidic stress, but HSP27 or αB-crystallin suppress this stiffening. The two sHSPs do not bind to the sarcomeres of stretched cardiomyocytes expressing very compliant titin (Ig domain-unfolding probability very low), but localize to the I-band region in stretched cardiomyocytes expressing stiff titin (Ig domain-unfolding probability high). In diseased human muscle/heart tissue both sHSPs associate with the titin springs, in contrast to cytosolic/Z-disk locations in healthy muscle/heart. We conclude that aggregation of unfolded titin Ig domains under stress conditions would stiffen myocytes, but sHSPs translocating to these domains prevent titin aggregation and cell stiffening.

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Individual Globular Domains and Domain Unfolding Visualized in Overstretched Titin Molecules

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Titin is a giant elastomeric protein responsible for the generation of passive muscle force. Mechanical force unfolds titin's globular domains, but the exact structure of the overstretched titin molecule is not known. Here we analyzed, by using high-resolution atomic force microscopy, the structure of titin molecules overstretched with receding meniscus. The axial contour of the molecules was interrupted by topographical gaps with a mean width of 28.5 nm that corresponds well to the length of an unfolded globular (Ig or FN) domain. The wide and apparently multimodal gap-width distribution suggest, however, that additional mechanisms such as partial domain unfolding and the unfolding of neighboring domain multimers may also be present. In the folded regions we resolved globules with an average spacing of 5.9 nm, which is consistent with a titin chain composed globular domains with extended interdomain linker regions. Topographical analysis allowed us to allocate the most distal unfolded titin region to the kinase domain, suggesting that this domain systematically unfolds when the molecule is exposed to overstretching forces. The observations support the prediction that upon the action of stretching forces the N-terminal ß-sheet of the titin kinase unfolds, thus exposing the enzyme's ATP-binding site and hence contributing to the molecule's mechanosensory function.

814-Pos Board B569

Interactive Properties of A-Band Titin

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The giant protein titin (chain weight ~3 MDa) has important roles in assembly and contractile function in vertebrate striated muscle sarcomeres. The molecule consists principally of ~300 Ig and Fn3 domains in a chain of more than 1 μm long spanning half the sarcomere. The A-band part is attached to the thick (myosin) filament with six molecules in each half filament. The I-band part makes an elastic connection between the tip of the filament and the Z-disc. The central region of A-band titin (C-zone, ~0.5 μm long) contains 11 copies of what is called the large super-repeat, **Ig**-Fn3-Fn3-**Ig**-Fn3-Fn3-Fn3-**Ig**-Fn3-Fn3-Fn3. The lengths of the large super-repeat (~43 nm) and of its sub-periods (~14 nm) correspond to the two main periodicities of the thick filament. C-zone titin is thought to make at least three distinct types of interactions: with itself, with myosin, and with myosin binding protein-C. We studied three recombinant 2-domain fragments from the three sub-periods of a large super-repeat. At physiological ionic strength all three constructs were soluble and mostly monomeric, whereas reduced salt resulted in self-association. Self-association was strongest in the case of the third sub-period construct leading to its complete sedimentation. Electron microscopy revealed large net-like oligomers