behavior. In addition, we show that NaK undergoes a voltage-dependent inactivation process, which is functionally similar to that seen in K⁺ channels. This inactivation may contribute to the low flux of Rb⁺ through NaK. Our functional characterization, along with the known crystal structures, now allows us to use NaK as a model system to further investigate structure-function correlations in non-selective channels and related selectivity filters.

3160-Pos Board B265
Engineering the hERG1 Selectivity Filter into the NaK Pore Domain

Comparison of the hERG1 selectivity filter sequence and pore helix with a variety of prokaryotic ion channels revealed an unusually high sequence identity (63%) with the non-selective cation channel NaK (~19 amino acids). Taking advantage of this remarkable similarity, we have used NaK as a template to carry out a structural analysis of a hERG1-like NaK selectivity filter in K-+. To engineer the NaK filter into that of hERG1, we substituted two critical residues, V959 in the pore helix and D66G at the selectivity filter (equivalent to positions S620 and P627 in hERG1, respectively). The final construct shares 73% sequence identity with the hERG1 selectivity filter, equivalent to the closest orthologue of hERG1, the bovine ether-a-go-go channel (bEAG1). Crystals of this engineered hERG-NaK diffracted to 2.8 Å resolution and were solved by molecular replacement using closed NaK as template. The crystal structure of hERG-like NaK in 100 mM K-+ revealed a striking similarity to the all canonical K-+ channel filters in the conductive conformation (RMSD=0.5 Å with the KcsA filter) and shows three major differences compared to WT NaK. First, hERG-like NaK filter revealed four equivalent K-+ binding sites. Second, the side chain of Phe66 residue establishes critical packing interactions with the adjacent aromatic residues from the pore helix. Third, hERG1-like NaK shows hydrogen bond interactions through a water molecule behind the selectivity filter, which is absent in WT NaK, but present in KcsA. We suggest that the hERG-NaK construct represents a unique tool to investigate the properties of the hERG1 channel selectivity filter at atomic level.

Muscle: Fiber and Molecular Mechanics & Structure II

3161-Pos Board B266
Radial Motion of Myosin Heads in Isolated Intact Rat Myocardium in Diastole

The main cellular mechanism that underlies the so-called “Frank-Starling Law of the Heart” is an increase in the responsiveness of cardiac myofilaments to activating Ca²⁺ ions at longer sarcomere lengths (SL). The fundamental mechanism responsible for this increase in responsiveness has been elusive, despite considerable experimental scrutiny. Here we tested the hypothesis that the increase in calcium sensitivity upon increasing SL is correlated with a radial outward movement of the myosin heads during diastole. 2D x-ray diffraction patterns were obtained from electrically stimulated intact, twitching papillary muscle isolated from rat hearts during a 10 ms time window in diastole just prior to electrical stimulation. A range of sarcomere lengths was compared either at Lmax (SL= ~2.3 μm) or following a quick release to slack length (SL= ~1.9 μm). The relative position of myosin heads was first assessed by the I₁/I₁₀ equatorial intensity ratio. To our surprise, I₁/I₁₀ was negatively correlated with SL, i.e. I₁/I₁₀ was less at Lmax vs. slack length. A more direct measure of the radial position of the myosin heads can be estimated from the position of the first maxima on the unsampled myosin layer lines, which are prominent in diastole. The intensity maxima, when examined pair-wise, moved prior to electrical stimulation. A range of sarcomere lengths was compared either at Lmax (SL= ~2.3 μm) or following a quick release to slack length (SL= ~1.9 μm). The intensity maxima, when examined pair-wise, moved prior to stimulation. A range of sarcomere lengths was compared either at Lmax (SL= ~2.3 μm) or following a quick release to slack length (SL= ~1.9 μm).

3162-Pos Board B267
Electron Tomography of Thick Sections of Insect Flight Muscle

Insect flight muscle (IFM) is a good model system within which to visualize actin-myosin interactions due to its highly ordered lattice of actin and myosin filaments. Lepidopteran flight muscle is perhaps the best ordered muscle in nature. Electron tomography (ET) of Lethocerus IFM has recently resulted in a model for the weak to strong transition that incorporates large azimuthal changes in the position of the lever arm compared to that predicted from crystal structures of myosin subfragment 1 in both the nucleotide free and transition states (Wu et al. PLoS-ONE, Sept. 2010). Those studies did not visualize the S2 domain in either the raw tomogram or in subvolume averages which would clarify the crossbridge origin. Here we have used ET of IFM fibers in rigor in which the filament lattice has been swollen in low ionic strength buffer to view where S2 emerges from the thick filament backbone as a test of the weak to strong transition. Previous ET on myac layers (single filament layers containing alternating myosin and actin filaments) of these same swollen rigor fibers revealed the S2 domain with clarity. In the present work, we are examining 80 nm thick transverse and longitudinal sections of swollen rigor IFM fibers in order to visualize all of the crossbridges originating from each 14.5 nm crown on the thick filament, but especially the so-called lead bridges, which bind the thin filament within the same target zone of isometric contraction. Class averages of both thick filaments as well as myac layers are being pursued. The thick filaments show subfilaments in the backbone and many of the myac layer raw repeat subvolumes show S2. Progress on this study will be presented. Supported by NIGMS and NIAMS.

3163-Pos Board B268
Vanadate Responses of Insect Flight Muscle

When exposed to sodium orthovanadate (Vi), permeabilized insect flight muscle (IFM) from Lethocerus water bugs behaves differently in three ways from vertebrate skeletal muscle (VSKM) under similar conditions. Weak binding of Vi in RLX: IFM treated 5-10 min with 250 μM Vi in Lo-μ M Vi-NaK buffer (pCa ~9.0, μ = ~90 mM), then washed free of Vi in Lo-μ M RLX, and placed in Lo-μ M ACT buffer (pCa ~4.5; μ = ~105 mM) rises to maximal isometric tension far more slowly (fives of minutes) than untreated IFM, suggesting a weak-binding, slowly reversible interaction with (presumably) myosin.

IFM 100x more sensitive to Vi than VSKM: Exposing maximally Ca-activated IFM to 250 μM Vi in Lo-μ M ACT (= Vi-ACT) quickly suppresses active-state tension 97-100% and induces relaxed-state X-ray diffraction structure. Vi-ACT-exposed IFM recovers little or no active-state tension during 30-60 subsequent minutes in Lo-μ M ACT. Vi-trapping in IFM is so strong that just 2 μM Vi ultimately (30-50 min) suppresses active tension in Lo-μ M ACT by ~75%, suggesting that 50% IFM force inhibition would require 1 μM or less Vi, versus 45-94 μM in VSKM.

Only crossbridges opposite target zones are Vi-trapped: Despite 97-100% paralysis of Ca-activated isometric force production by Vi-ACT, Vi trapping only affects myosin crossbridges opposite actin target zones. When stretched 2-4%, fibers recover active tension capability. Post-Vi washout with RLX, followed by 2-4% stretch, followed by Lo-μ M ACT exposure, generates significant Ca-activated tension, because at the longer sarcomere length target zones have moved toward myosin heads that at rest length were unable to reach the 2/3 front of IFM actin monomers (Wu et al., PLoS One 5: e12643 (2010)) that accept strong-binding crossbridges. ATpase cycling and tight Vi trapping. Slow 3% length-cycling in Vi-ACT Vi-traps all accessible IFM crossbridges. (Support: NIH, MDA.)