the C-terminal domain, thus causing no activation of ATPase activity. With the addition of 1 μM NEMS-1 the ATPase activation decreased from ~ 225% at pCa 7.5 to ~ 85% at pCa 6.5. The data suggest that in the absence of Ca^{2+} at site II strong-binding myosin crossbridges cause opening of more active sites on the thin filament if the C-domain is occupied by Mg^{2+} rather than Ca^{2+}. This effect could be relevant to the contraction-relaxation kinetics of cardiac muscle. As Ca^{2+} dissociates from site II during the relaxing phase of the cardiac cycle, residual Ca^{2+} bound at the C-terminus might facilitate the switching off of the thin filament and the detachment of crossbridges from actin.

612-Pos Board B412
The Influence of Troponin C Isoforms on Drosophils Stretch Activation and Power Generation
Catherine J. Eldred, Laura Koppes, Kevin Georgek, Andrea Page-McCaw, Belinda Bullard, Douglas M. Swank.
Drosophila indirect flight muscle (IFM) is one of the most rapidly contracting muscle types known. To efficiently generate power for flight at such high speeds, insects have evolved stretch activation and shortening deactivation. In IFM of some insects, in addition to a typical calcium binding troponin C, TnC1, there is a unique, additional isoform, TnC4. TnC1 has 2 calcium binding sites, a high-affinity structural site and a lower-affinity regulatory site. TnC4 has only the high-affinity structural binding site. It is hypothesized to respond to stretch rather than Ca^{2+} concentration to further activate the thin filament. We are investigating TnC4 in *Drosophila* via two different routes. The first is to chemically remove TnC from IFM using either o-vanadate or trifluoperazine which eliminates power generation and stretch activation. Bacterially-expressed TnC1 and TnC4 are reincorporated into the fiber in physiological proportions, which restores power-generating ability and stretch activation to the IFM. We are currently optimizing the extraction and reintroduction protocol to determine the effects on stretch activation of varying the ratio of TnC1 and TnC4. The second method is to breed a TnC4 null using p-element mediated male homologous recombination. We are performing this by crossing flies containing identical p-elements in positions which bracket the TnC4 gene on chromosome 2R. When the p-elements are mobilized, some of the offspring will have the region between the p-elements excised. We are currently screening offspring of the final cross for null TnC4 alleles. These two methods of investigation into TnC4 function will allow modified TnC4 to either be introduced or expressed in IFM to help elucidate TnC4's structural mechanism.

613-Pos Board B413
Cross-Bridge Populations and the Biphasic Time Course of Muscle Relaxation
Julien S. Davis, Neal D. Epstein.
A biphasic time course of relaxation follows Ca^{2+} removal from a contracting muscle fiber–a small linear decline is followed by a faster large exponential drop in tension to the relaxed state. Our aim is to see if recent insights into tension generation and the cross-bridge cycle can shed light on these kinetics (Davis and Epstein, 2009). In fully activated isometric rabbit psoas fibers two distinct and roughly equal populations of myosin heads are attached to actin. Half are termed ''competent'' and are capable of generating tension, while the other half are termed ''noncompetent'' and serve to ''buffer'' tension. For example, a step-decrease in fiber length releases a steric constraint on noncompetent cross-bridges thereby triggering tension recovery.
We investigate whether the ''buffer'' of noncompetent AMD bridges might support the linear phase, with the exponential decline ($k_{REL\ FAST}$) mediated by the decay of the remaining competent cross-bridge population. To do this, the linear phase was treated as a steady-state reaction with *duration* and not slope used to determine $k_{REL\ SS}$. We found $k_{REL\ SS}$ normalized to k_{CAT} (fiber ATPase) if a reasonable ~21% of myosin heads constitute the noncompetent buffer. This leaves the final exponential decline to the relaxed state governed by the discharge of tension generating competent cross-bridges on exhaustion of the noncompetent buffer population. Additional support comes from the observations that added P_i decreases the duration and increases the slope of the linear phase with virtually no effect on $k_{REL\ FAST}$ (Tesi *et al,* 2002), and that a slow Tn Ca^{2+} off switch prolongs the linear phase (Kreutziger *et al,* 2008). Arrhenius plots of k_{CAT}, $k_{REL\ SS}$ and $k_{REL\ FAST}$ are similar in slope, suggesting a common rate-limiting step of strain-sensitive ADP cross-bridge dissociation for all processes.

614-Pos Board B414
Single Molecule FRET Measurements Reveal Incomplete Activation of the Cardiac Myofilament by Ca2+
John M. Robinson, Maria E. Moutsoglou, Troy Lackey, Wenji Dong, Mathivanan Chinnaraj, Herbert C. Cheung.
We report pulsed interleaved single pair FRET (spFRET) measurements of freely diffusing reconstituted cardiac myofilaments. Under saturating Ca2+ conditions, the measurements reveal a population of unactivated troponin. We suggest that this population of unactivated troponin comprises a form of cardiac reserve that is regulated by signaling pathways that target the myofilament. The results are interpreted in terms of a coarse-grained free energy landscape model of myofilament activation.

615-Pos Board B415
Structural Dynamics of the C-Domain of Cardiac Troponin I In the Reconstituted Thin Filament
King Lun Li, Zhiqun Zhou, Daniel Rieck, Yexin Ouyang, **Wenji Dong**.
The inhibitory region (Ir), regulatory region (Rr), and mobile domain (Md) within the C-domain of cardiac troponin I (cTnI) play different functional roles in regulating the thin filament switch. The functional role of each region is believed to be associated with structural dynamics of the region. This study used fluorescence polarization to acquire dynamic information on the C-domain of cTnI in the reconstituted thin filament. To achieve this goal, a series of cTnI mutants were generated with single-cysteine at residues 131, 145, and 151 within the Ir, residues 160 and 167 within the Rr and residues 177, 182, 188, 200, and 210 within the Md. In addition, double-cysteine cTnI(177C/188C) and cTnI(182C/200C) were also generated to investigate the conformational change of the Md in response to Ca^{2+} regulation using homo-FRET. These cTnI mutants were labeled with fluorophore TAMRA and reconstituted with other proteins to form thin filament. The reconstituted samples were subjected to steady-state and time-resolved fluorescence anisotropy measurements, and fluorescence quenching experiments under different biochemical conditions. The anisotropy measurements showed that the Ir was less dynamic than the Rr and Md at both Mg^{2+} and Ca^{2+} states. Ca^{2+} binding to troponin induced minimal changes in Ir dynamics, but significantly decreased Rr flexibility, and resulted to a more compact Md with more dynamics as whole. Furthermore, Cys210 at the C-terminus of cTnI was highly immobilized comparing to other segments of the domain in the Mg^{2+} state, and became highly dynamic in the Ca^{2+} state, suggesting a unique role of the C-terminus in regulating thin filament switching. Fluorescence quenching results, the effects of the strongly-bound S1 on the structural dynamics of the C-domain of cTnI, and a detailed data analysis will be discussed in this presentation.

616-Pos Board B416
Functional Characterization of the Last 5 Residues of the C Terminus in Cardiac Troponin I
Qian Xu, Shannamar Dewey, Susan Nguyen, **Aldrin V. Gomes**.
The C-terminus of cardiac troponin I (cTnI) is a highly conserved region of the protein. Previous reports have suggested that the last 17 residues at the C-terminal end of cTnI do not directly interact with cardiac troponin C (cTnC). However, a 17 residue C-terminal deletion in human cTnI is associated with myocardial stunning, and was previously found to increase calcium sensitivity in an in vitro motility assay (Foster et al., Circ. Res. 2003;93:917-924). To further investigate this region of cTnI, we generated three C-terminal deletion mutations in human cTnI: del1 (deletion of residue 210), del3 (deletion of residues 208-210), and del5 (deletion of residues 206-210). A monocysteine mutant of cTnC (C35S) was purified and labeled with the fluorescent probe 2-[4'-(iodoacetamido) anilino] naphthalene-6-sulfonic acid (IAANS) at Cys-84. Upon reconstitution of the labeled cTnC with cardiac troponin T (cTnT) and truncated or wild-type cTnI to form troponin complexes, the calcium-dependent changes in fluorescence were measured. The results show that the troponin complex with the cTnI del5 mutation had increased calcium affinity ($P<0.05$); while the cTnI del1- and del3 troponin complexes showed no significant difference in calcium affinity when compared to wild-type troponin. Mammalian two-hybrid studies showed that the interaction between cTnC and cTnI deletion mutants were impaired in del3 and del5 mutants when compared to wild-type cTnI. Two-hybrid studies also showed that the interaction between cTnT and the cTnI del5 mutant was impaired when compared to wild-type cTnI or the other deletion mutants. Our results suggest that the last 5 C-terminal residues of cTnI are important for the physiological functions of cTnI and directly influences the binding of cTnI with cTnC and cTnT.

617-Pos Board B417
Kinetics of Smooth Muscle Acrylodan-Tropomyosin Transitions on Actin
Evan Lutz, Mechthild M. Schroeter, Tamatha Baxley, **Joseph M. Chalovich**.
Actin-tropomyosin generally has the potential to exist in states that are active, inactive or intermediate with respect to activation of myosin S1-ATPase activity. S1 stabilizes the active state while proteins that inhibit ATPase activity may function by stabilizing the inactive state. The transition among states can be initiated by rapidly dissociating S1 from actin-tropomyosin with ATP. We tested the ability of acrylodan labeled smooth muscle tropomyosin to monitor changes in the presence of skeletal muscle troponin. With saturating calcium there was a rapid decrease in acrylodan fluorescence as the actin-tropomyosin made the