and attached heads is similar. Supported by Ente Cassa di Risparmio di Firenze, FIRB-Futuro in Ricerca, MRC and ESRF.

734-Poo Board B520
Role of Pro-Ala-Rich Extension of Troponin in Insect Flight Muscle as Examined by X-Ray Diffraction
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The mechanism of stretch activation is essential for the asynchronous action of insect flight muscle (IFM). Our previous study has shown that the 1.1 row-line spot on the 1st layer line reflection from bumblebee IFM is the first one to respond to the stretch signal in a calcium-dependent manner, and the model calculation has suggested that it may come from the structural change of troponin. This makes IFM troponin a candidate for the stretch sensor. Then the question is what structure transmits stretch information to troponin. IFM troponin-I (troponin-H) is known to have a long Pro-Ala-rich extension, and this may extend to the thick filament and pick up the stretch information. To test this possibility, the extension was severed by a specific endoprotease (Igase) in bumblebee IFM, and its mechanical properties and X-ray diffraction patterns were examined. In honeybee troponin-H, the susceptible sequence is located at the base of the extension. Unexpectedly, the ability of stretch activation was not affected. The severing had a dramatic effect on diffraction patterns: In IFMs in general, the equatorial 1.0 row-band reflection is much more intense than the 1.1 reflection, but after Igase treatment, the intensities of these reflections became almost equal. If this change is caused by the reduced mass of the thin filament, its mass should be reduced to 1/4 while the extension accounts for only 10% of the thin filament mass. In the SDS gel pattern, the 80-KDa band for troponin H disappeared from the fiber and several protein bands appeared in the solution after treatment. A possibility is that each extension binds several GST molecules (Clayton et al., 1998) to process reactive oxygen generated by the high mitochondrial activities in IFM cells.

735-Poo Board B521
Modeling the Working Stroke of the Muscle Crossbridges
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It has been suggested by Knupp et al. (J.Mol.Biol. 390:168–181, 2009) that the lever arms of the myosin crossbridges (subfragment 1) make virtually no contribution to the behavior of the M6 meridional reflection, and that recent X-ray interference studies provide no evidence for lever arm tilting during the working stroke. Instead, it is claimed that the observed changes can be predicted by models in which the working stroke involves a length change of subfragment 2, with the lever arm remaining in either the Dominguez or Rayment configurations (the conventional pre- and post-working stroke positions), or by models in which a changing mixture of these configurations is present during the stroke.

In our previous work (Huxley et al. J.Mol.Biol. 353:743–761, 2006), we showed that the relative intensities of the M3 and M6 reflections and of the inner and outer interference peaks of M6 require that in isometric contraction the crossbridge lever arms must be angularly dispersed through ±20° or more, and that a second component, presumably in the myosin backbone, must contribute to M6 to shift the phase of the interference pattern appropriately. The observed steady increase in the M6 intensity, by 30–40% or more, with releases up to ~7 nm, and then a small decrease, is modeled satisfactorily with the tilting lever arm. However, if the modeling involves a fixed lever arm angle (either Dominguez or Rayment, or a mixture of the two configurations) with an S2 of varying length, the predicted M6 reflection undergoes several large swings in intensity over the relevant range of releases, and bears no relation to what is observed. This shows that the lever arm configuration plays a major role in determining the M6 behavior, and that the data support a straightforward tilting lever arm model.

736-Poo Board B522
Length Dependent Activation in Synchronous Flight Muscle from Manduca Sexta
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In most striated muscles, the amount of force generated at a given concentration of activating calcium is greater at long sarcomere length (SL) than at shorter SL, referred to as “length dependent activation” (LDA). LDA is prominent in mammalian cardiac muscle and underlies the so-called Frank-Starling Law of the Heart” allowing cardiac output to be adjusted on a beat-to-beat basis. The molecular basis for LDA is not yet understood. The dorsal longitudinal flight muscle (LM) of the Hawkmoth Manduca sexta is an emerging model system for structural and functional studies of muscle. It is a synchronous muscle requiring a neural impulse for every muscle twitch, as in skel- etal and cardiac striated muscle, but it is structurally similar to the more widely studied asynchronous insect flight muscles of Drosophila and Lethocerus. Its force-length curve has been shown to be remarkably similar to mammalian car- diac muscle (Tu & Daniel, J Exp Biol 207: 2455. 2004) indicating that Man- duca flight muscle might be a useful model system to elucidate various aspects of cardiac function in comparative studies. The present studies were un- dertaken to characterize LDA in Manduca flight muscle. Conditions were found that allowed chemical skinning of the muscles while maintaining good structural order as assessed by bright and X-ray diffraction. Force-pCa curves were collected as a function of SL. Dorsally located DLMs (cooler in vivo) were compared to ventrally located DLM’s (warmer in vivo). We found that both dorsal and ventral DLMs show length-dependent activation. Our study also showed that ventrally located DLM’s are less cooperative (Hill coefficient nH ~1-1.2) than the dorsally located DLM’s (nH ~1.8-1.9) which may be related to their different functions in vivo. Supported by NSF IOS 1022058 and NIH RR08630.

737-Poo Board B523
The Origins of Cross-bridges in Active and Rigor Insect Flight Muscle are not As Predicted from Acto-S1 and X-Ray Crystallography
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In fast-frozen, actively contracting insect flight muscle (IFM) fibers we noted a surprising azimuthal slew in the myosin lever arms that was wholly unex- pected from the currently available crystal structures of isolated myosin S1 and which indicates the crystal structures may not reflect the normal in situ constraints within the sarcomere where myosin cross-bridges are restricted to originate from a well defined zone on the thick filament [Wu et al., 2010, PLoS-ONE, 5(9): e12643]. However, previous studies did not reveal the S2 domains, which most accurately define the myosin head origin. Here, we have used electron tomography (ET) of Lethocerus IFM fibers in rigor in which the filament lattice has been swollen in low ionic strength buffer to view the S2 origins of rigor “lead bridges”. These rigor lead bridges bind to the same region of the thin filament as myosin heads of active muscle so their origins should reflect the same thick filament positions as active heads. We examined 80 nm thick transverse sections cut with a vibrating knife to minimize section compression and shearing artifacts, and imaged with < 60 e-/Å² total exposure during each tilt series to minimize radiation-induced section thinning. This analysis shows two different distributions of lead bridge origins depending on the presence of the rear bridges. However, the origins are consistent with target zone accessibility of strong binding myosin heads being limited to two successive 14.5 nm crowns. Subvolume averages of both thick filament as well as actin filaments are being pursued with the goal of reassembling a region of the filament lattice using high S/N averages. Supported by NIGMS and NIH.

738-Poo Board B524
Interaction Between the Relay Loop and the SH1-SH2 Helix Region in Drosophila Muscle Myosin is Essential for Normal Motor Function, Myofibril Stability and Muscle Contraction
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Department of Biology, Molecular Biology Institute, and SDSU Heart Fibril Stability and Muscle Contraction
Drosophila Muscle Myosin is Essential for Normal Motor Function, Myofibril Stability and Muscle Contraction

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Molecular modeling of Drosophila muscle myosin reveals that relay domain residue E499 interacts with SH1-SH2 residue R714 in a charge-dependent manner. To explore the significance of this interaction, we generated trans- }