cells of the mouse cerebral cortex in acute brain slices. VSFP2.3 expression in neocortical cells was achieved by *in-utero* electroporation into the cortical ventricular zone at embryonic age 15.5 of a plasmid vector containing VSFP2.3 under the CAG hybrid promoter. This procedure resulted in strong VSFP2.3 fluorescence at postnatal age (up to day 30 tested) from a restricted cortical area, mostly within somato-sensory cortex, with the fluorescence originating from a clustered population of pyramidal neurons with cell bodies in layer 2/3. Electric current injection into VSFP2.3-positive cells (postnatal day 16-22) revealed an optical response signal to sub-threshold slow depolarization of the somatic membrane that could be resolved in single trials. While the optical signal in response to fast action potentials was noisy in single trials, S/N above two was obtained by event-triggered averaging over a few (5-10) action potentials. We also tested for the optical response to synaptically evoked EPSPs which were reliably detected at near threshold amplitudes in single trials. Our results provide the first demonstration of an optical readout of neuronal activity at cellular resolution using a genetically-targetable voltage probe in intact brain tissue *in-vitro*.

1119-Plat
Functional and Structural Characterization of A New Monomeric Far-Red Fluorescent Protein
Michael Z. Lin[1], Michael R. McKeown[2], Ho Leung Ng[3], Tom Alber[3], Roger Y. Tsien[2].
[1]Stanford University, Palo Alto, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA, [3]University of California, Berkeley, Berkeley, CA, USA.
Fluorescent proteins have become valuable tools for biomedical research as protein tags, reporters of gene expression, biosensor components, and cell lineage tracers. However, applications of fluorescent proteins for deep tissue imaging have been constrained by the opacity of tissues to excitation light below 600 nm, due to absorbance by hemoglobin. Fluorescent proteins that excite efficiently in the "optical window" above 600 nm are therefore highly desirable. We report here the evolution of a far-red fluorescent protein with peak excitation at 600 nm and peak emission at 650 nm. This, Neptune, performs well in imaging deep tissues in living mice. The crystal structure of Neptune reveals novel mechanisms for red-shifting, including the acquisition of a new hydrogen bond with the chromophore. Neptune may serve as the basis for fluorescent indicators or FRET reporters that are more compatible with deep tissue imaging.

1120-Plat
Adaptive Phase Modulation for Multiphoton Microscopy
Rebecca M. Williams, Warren R. Zipfel.
Cornell University, Ithaca, NY, USA.
Tissue structures present index mismatches at a variety of spatial scales that can aberrate the focal volume and thus blur cellularly resolved multiphoton images acquired within biological tissues and live animals. We are investigating the extent to which adaptive phase modulation can be used to reconstruct the point-spread-function (PSF) and enable deeper and clearer multiphoton imaging into biological tissues. To do this, a Ti:Sapphire beam is reflected off of a reflective spatial light modulator conjugate to the objective pupil plane. The excitation PSF is directly imaged with a separate objective mounted perpendicular to the optic axis. We find that the fluorescence signal increases with increasing size of the scattering structures. Resolution degradation, however, reaches a maximum with scatterer spatial frequencies at one tenth of the maximal frequency allowed by the focusing objective NA. PSF aberrations from tissue structures can be somewhat compensated by modulating the phase at the back aperture using Zernike polynomials as a basis set for increasing overall image brightness. In this scheme two fitness measurements are required for each Zernike order. Initial results through tissue sections show that spherical aberration is a problem, but not the only problem. (Research supported by NIH/NCI R01 CA116583.)

Platform T: Muscle: Fiber & Molecular Mechanics & Structure II

1121-Plat
Relay Loop Stabilizes the Force-Generating Region in Myosin
Yuri E. Nesmelov[1], Roman V. Agafonov[2], Igor V. Negrashov[2], Sarah Blakely[2], Margaret A. Titus[2], David D. Thomas[2].
[1]University of North Carolina, Charlotte, NC, USA, [2]University of Minnesota, Minneapolis, MN, USA.
We have used transient time-resolved FRET (TR^2FRET) to monitor the conformation of the relay helix in a myosin II functional mutant during the recovery stroke in real time. Myosin was perturbed with the F506A mutation (*Dictyostelium discoideum* sequence), located within the relay loop in the force-generating region. F506 is a highly conserved residue in myosin II and is a hypertrophic cardiomyopathy mutation site. Previous studies [Tsiavaliaris, EMBO Rep, 2002, 3(11), 1099] showed a significant effect of the F506A mutation on myosin function. Actin affinity in the presence of ATP was increased, and the mutant did not move actin filaments in *in vitro* motility assays. A small decrease in intrinsic fluorescence was observed upon addition of excess ATP, but ATP binding and hydrolysis were not affected by the mutation. It was proposed that the F506A disrupts the communication between the active site and the lever arm. We engineered a double-Cys myosin mutant (A639C:K498C) in the Cys-less background with the F506A functional mutation, and labeled the mutant with optical probes. We used TR-FRET to determine the interprobe distance, and TR^2FRET measurements after rapid mixing with ATP revealed changes in the relay helix conformation during the recovery stroke in real time. The mutation induced significant disorder of the relay helix in the force-generating region, but myosin still produces a recovery stroke, changing the relay helix conformation from straight to bent. We conclude that (a) the relay helix is disordered in myosin functional mutant F506A, which demonstrates the importance of the relay loop - relay helix interaction in the relay helix stabilization, and (b) the relay helix is the major structural element in the force-generating region of myosin, responsible for communication from the active site to the converter domain and the lever arm.

1122-Plat
Converter Domain Residue R759 Interaction with Relay Loop Residue N509 in Drosophila Muscle Myosin is Critical for Motor Function, Myofibril Stability and Flight Ability
Girish C. Melkani, William A. Kronert, Anju Melkani, Sanford I. Bernstein.
Department of Biology, Molecular Biology and SDSU Heart Institute, San Diego State University, San Diego, CA, USA.
We used an integrative approach to probe the significance of the interaction between the relay loop and converter domain of Drosophila melanogaster skeletal muscle myosin. We generated a transgenic line expressing myosin with a mutation in the converter domain (R759E) at the relay loop interaction site. The mutation depresses calcium, basal or actin-activated MgATPase values (Vmax) by ~60% and actin sliding velocity ~35% compared to wild-type myosin. Ultrastructure of two-day-old adult fibers shows cracking and frayed myofibrils with some disruption of the myofilament lattice which becomes more severe in one-week-old adults. Flight ability is reduced in two-day-old flies compared to controls and is absent in 1-week-old adults. Thus appropriate interaction between the relay loop and converter domain is essential for normal motor function, myofibril stability and locomotion. To examine the specificity of this interaction, we used a compensatory mutational approach to attempt to restore the function of the R759E mutant myosin. Our modeling indicates that relay loop residues N509 and D511 interact with converter domain residue R759. To verify our model, we generated two transgenic lines that express R759E and either the N509K or D511K mutations. Interestingly, calcium, basal, and actin stimulated ATPase values are restored to 70% and actin sliding velocity is restored to 90% in N509K/R759E but not in D511K/R759E. Structurally fibers from 2-day or one-week -old adults appear morphologically normal in N509K/R759E and their flight ability is like wild type. However, D511K/R759E myofibrils do not show any improvement compared to R759E and flight ability is worse than R759E. Overall, our results reveal the critical interaction between the converter domain with relay loop residues and their role in myosin motor function and myofibril assembly/stability.

1123-Plat
Familial Hypertrophic Cardiomyopathy Mutations of the Myosin Regulatory Light Chain Remove Myosin Load Sensitivity
Michael J. Greenberg[1], Katarzyna Kazmierczak[2], Danuta Szczesna-Cordary[2], Jeffrey R. Moore[1].
[1]Boston University, Boston, MA, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.
The myosin head domain consists of a globular head and an elongated alpha-helical neck region, the "lever arm", which undergoes large conformational changes during the ATPase cycle. This lever arm has been proposed to be part of the communication pathway transmitting external loads to the active site. Since the regulatory light chain (RLC) supports and imparts stiffness to the myosin lever arm, we hypothesized that alterations in the structure of the myosin heavy chain-RLC interaction could alter myosin load-dependent biochemistry.

Using modifications to the *in vitro* motility assay under unloaded and loaded conditions, we examined the biochemical properties of two RLC mutations that have been implicated in causing familial hypertrophic cardiomyopathy, N47K and R58Q. Myosin was purified from porcine ventricles carrying beta-MHC, and the native RLC was replaced with recombinant human wild type (WT) or mutant RLC. Our data show that under unloaded conditions, there are no differences between the mutant myosins and the WT. On the other hand, consistent with skinned fiber studies, we saw significant changes under loaded conditions, with both mutants showing reductions in isometric force, power output, and the load at which peak power occurs. We also see that both ATP and ADP affinity are load-dependent in the WT. Interestingly, our data suggests that cardiac myosin undergoes a load-dependent isomerization in the ADP bound state, similar to other "load sensing" myosins. Furthermore, we show that whereas WT shows a reduction in affinity for exogenously added ADP under loaded conditions, the mutants are relatively insensitive to load. Taken together these data demonstrate that mutations of the RLC change the load dependent kinetics of cardiac myosin, suggesting a role for the RLC in tuning load-dependent myosin mechanochemistry.

1124-Plat

Gravitational Force Spectroscopy Reveals Separation of Myosin Heads at the S1/S2 Hinge

James Dunn, Kathy Wang, Irene Cai, Nakiuda Hall, **Douglas D. Root**.
University of North Texas, Denton, TX, USA.

In order for striated muscle myosin to interact productively with thin filaments, it must both span the gap between thin and thick filaments and possess sufficient degrees of freedom to align its actin binding site with protomers of various helical orientations. This flexibility requires extensions and rotations of the coiled coil S2 domain of myosin including the S1/S2 hinge. To examine the ability of the coiled coil to separate, a novel gravitational force spectrometer was created that can apply femtonewton to piconewton forces with high accuracy to regions of a single myosin molecule defined by site-specific antibodies. Force-distance curves indicate that when the piconewton forces are applied perpendicular to the long axis of the S2 coiled coil at the S1/S2 hinge, the strands separate readily in a force dependent manner. However when similar levels of force are applied parallel to the long axis of the S2 coiled coil, there is much less extension. Computational force spectroscopy simulations on the atomic model of human S2 provided confirming results and with atomic resolution detail. As a control, simulations on scallop myosin S2 indicated that its coiled coil separates with less force than human S2 which is consistent with previously published reports. Furthermore, several familial hypertrophic cardiomyopathy causing mutations in the S1/S2 hinge were introduced into the human S2 simulations and resulted in further destabilization of its nanomechanical properties. These results indicate that the myosin S2 has intrinsic structural functions that may be independent of its interactions with other proteins. It is possible that interactions with myosin binding proteins may modulate these properties. (Supported by NSF 084273 ARRA)

1125-Plat

Three-Dimensional Structure of the Relaxed State of Calcium-Regulated Myosin Filaments

John L. Woodhead, Fa-Qing Zhao, **Roger Craig**.
UMass Medical School, Worcester, MA, USA.

Myosin filaments of muscle are regulated either by phosphorylation of their regulatory light chains or Ca^{2+}-binding to the essential light chains, contributing to on-off switching or modulation of contraction. Phosphorylation-regulated filaments in the relaxed state are characterized by an asymmetric interaction between the "blocked" and "free" heads of each myosin, inhibiting actin-binding or ATPase activity (Wendt et al., 2001; Woodhead et al., 2005). We have tested whether a similar interaction occurs in Ca^{2+}-regulated filaments. Filaments were purified from scallop striated adductor muscle and imaged by cryo-electron microscopy. 3D reconstruction was carried out by single particle methods. Reconstructions showed a 7-fold symmetric, helical array of myosin head-pair motifs lying above the filament surface. Fitting of the motif with a myosin head atomic model revealed that the heads interact in a similar way to phosphorylation-regulated filaments. However, the 2-headed motif was more tilted and higher above the filament surface in the Ca^{2+}-regulated filaments. Subfragment 2 of the myosin tail emerged from the motif near the blocked head and connected the motif to the filament backbone, which comprised a 7-fold array of comma-shaped subfilaments. This structure reveals new detail compared with a previous cryo-EM study (Vibert, 1992) and demonstrates that the interpretation of head organization in a negative stain reconstruction of scallop filaments (AL-Khayat et al., 2009) is incorrect. We conclude that the relaxed state of Ca^{2+}-regulated filaments is achieved in a similar way to phosphorylation-regulated filaments, confirming that head-head interaction is a widely used motif (Woodhead et al., 2005). In the scallop filament, the pairs of myosin heads are much closer together azimuthally and the subfilaments have a different structure compared with phosphorylation-regulated filaments, implying that general models for thick filament structure (Squire, 1973) need modification. Supported by NIH grant AR34711.

1126-Plat

Electron Tomography of Cryofixed, Isometrically Contracting Insect Flight Muscle Reveals Novel Actin-Myosin Interactions

Shenping Wu[1], Jun Liu[1], Mary C. Reedy[2], Hanspeter Winkler[1], Richard T. Tregear[3], Carmen Lucaveche[2], Clara Franzini-Armstrong[4], Hiroyuki Sasaki[5], Yale E. Goldman[4], Michael K. Reedy[2], **Kenneth A. Taylor[1]**.
[1]Florida State University, Tallahassee, FL, USA, [2]Duke University Medical Center, Durham, NC, USA, [3]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [4]University of Pennsylvania, Philadelphia, PA, USA, [5]Jikei University School of Medicine, Tokyo, Japan.

We have applied multivariate data analysis to 38.7 nm repeat segments obtained from electron tomograms of isometrically contracting insect flight muscle fibers, mechanically monitored, rapidly frozen, freeze-substituted and thin sectioned. Improved resolution reveals for the first time the helix of F-actin subunits in the thin filament with sufficient clarity that an atomic model can be built into the density independent of the myosin cross-bridges, thereby providing an objective method for identifying weak and strong actin-myosin attachments. The tomogram shows strong binding myosin attachments on only four F-actin subunits midway between successive troponin complexes; these actin subunits comprise the "target zone" of active contraction. Improved quantitation facilitates a more detailed description of weak and strong myosin attachments all along the thin filament including for the first time myosin heads contacting the thin filament on troponin. Most strong binding actin attachments consist of single myosin heads but 28% of bound heads are 2-headed myosin attachments. Strong binding attachments show an axial lever arm range of 77° sweeping out a distance of 12.9 nm. The azimuthal range for the lever arm of strong binding attachments is 127° with a distribution nearly completely to one side of the initial crystallographic structures used for the fitting. There is no apparent coupling between axial angle, representing progress through the power stroke of myosin, and the azimuthal lever arm angle. Two types of weak actin attachments are observed. One type, which is found exclusively on target zone actin subunits, appears to represent prepowerstroke intermediates. The other, which appears to have a different function, is positioned on the M-ward side of the target zone, i.e. the direction toward which filaments slide during sarcomere shortening. Its motor domain contacts tropomyosin rather than actin. Supported by NIH.

1127-Plat

Microscopic Measurement of Periodic Cross-Bridge Formation in Skeletal Myofibril

Madoka Suzuki[1,2], Shin'ichi Ishiwata[3,2].
[1]Comprehensive Research Organization, Waseda University, Tokyo, Japan, [2]Waseda Bioscience Research Institute in Singapore, Waseda University, Tokyo, Japan, [3]Department of Physics, Faculty of Science and Engineering, Waseda University, Tokyo, Japan.

Electron microscopy and X-ray diffraction studies have been usually used for the examination of the interactions between actin and myosin, i.e., cross-bridge formation, in muscle fibers. These studies suggested the existence of "target zones [regions]" in actin (thin) filament, which are composed of three to four actin monomers that myosin heads in myosin thick filament can form cross-bridges. Direct evidence for the target zones, however, has been missing in myofibrils. Here we studied the interaction between a single actin filament and the thick filaments of rabbit skeletal myofibril under optical microscope. Single bead-tailed actin filament was manipulated by optical tweezers to make rigor cross-bridges on the outer surface of myofibril, and rupturing events were directly detected. We found the periodic cross-bridge formation, and frequently observed gaps, which are probably due to the incommensurate helical pitches between the thin and the thick filaments.