showed that although local, near-to-equilibrium systems may be interlocked. Thus, for example, if the act of proton pumping were reversibly connected to ATP synthesis, the ATP could then be used as a key component of other near-to-equilibrium systems such as metabolism and/or cytoskeletal activities. I propose a specific mechanism for the act of proton pumping that qualifies as a near-to-equilibrium system. It embodies motion perpendicular to the membrane plane for ion pumping. The mechanism is both cooperative and synchronized. Each transport event results in a compaction of the protein across the membrane provoking a neighboring pump to expand and *visa versa*. The mechanism implies that pumps be dimers or multimers in living membranes although they could pump individually when reconstituted into bilayers. It is the nature of the pumping mechanism that it can be restarted with Brownian motion if it falters.

Platform U: Muscle: Fiber & Molecular Mechanics & Structure

1095-Plat
X-Ray Diffraction "Movie" Of Complete Oscillatory Work Cycles Myogenically Produced In Glycerinated Insect Flight Muscle (IFM)
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When slightly calcium-activated (pCa ~5.7, gives ~0.2 peak isometric force), glycerinated *Lethocerus* insect flight muscle (IFM) can be mechanically stretch-activated at constant [Ca^{2+}] to give a delayed active rise to peak force, a myogenic response typical of asynchronous IFM. Continuous sine-wave length-oscillations elicit sinusoidally cycling force traces, delayed ~45° behind length cycles. Force-length x-y plots therefore follow anti-clockwise Lissajous loops of elliptical form, enclosing an area proportional to oscillatory work output per cycle, which peaked at ~2%X~2Hz for 10-20-fiber bundles. A Pilatus 100K detector collected 64 synchrotron-x-ray fiber-diffraction frames per full cycle (8ms time resolution), throughout an 11-cycle run (704 frames). Summing successive cycles and adjacent frames produced a 16-frame movie (32ms time resolution) showing weaker details. The movie shows clear within-cycle peak-to-valley intensity changes in multiple reflections, some signaling crossbridge mass shifts toward (and away from) thin-filament lattice positions, others cross-bridge shifts between binding to and detaching from actin target zones, still others signaling crossbridge shifts between tilt angles mostly near 90° versus mostly dispersed to non-90° angles. Surprises include: 1) Maximum 90° angles occur near force peak in *Drosophila* but near force valley in *Lethocerus*. 2) Although the force sine-wave varies smoothly, two structural signals of crossbridge attachment show biphasic profiles as force rises and again as force falls, as if outer and inner myosin heads (AL-Khayat etal model) attach/detach in separate cohorts during the 2% (26 nm/half-sarcomere) length changes. 3) Structural signals of crossbridge action are variably phase-coupled to the force sine-wave; some x-ray signals even differ in phase lag between force peak and valley. Maximum tropomyosin shift spans ~5 frames around force peak. Overall results strongly constrain possible mechanisms of stretch-activation, suggesting complementary approaches for revealing it. (Support: NIH, DOE).

1096-Plat
Photoactivatable Quantum Dots in Super-Resolution 3D Microscopy of Myofibrils
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To image the relationships between immune-labeled myofibrilar proteins at sub-diffraction-limited resolutions, highly photostable quantum dots were chemically modified to make them photoactivatable. Although previous reports have used photoactivation of cyanine dyes and GFP variants for 2D super-resolution microscopy, photoactivatable quantum dots (PAQ dots) have sufficient brightness and photostability to enable 3D acquisitions of signals from individual quantum dots. The chemical synthesis of PAQ dots caused only minor changes in the spectroscopic properties and brightness of the activated PAQ dots relative to unmodified quantum dots as assessed by fluorescence lifetime imaging of single quantum dots. The PAQ dots were conjugated to Fab fragments for immunostaining of myofibrils. After optimizing conditions so that a balance between photoactivation and photobleaching of the PAQ dots occurred during 3D acquisition in a spinning disk confocal microscope, 3D images of individual quantum dots were reduced to the 3D center of mass and accumulated until sufficient data for a full image was generated. Initial results demonstrate sub-diffraction resolutions in XY and even more striking resolution improvements in Z. The superresolution images reveal finer structural details in the myofibrils than conventional confocal imaging. Unlike electron microscopy, all measurements are made in aqueous solutions. Furthermore, the ability to make PAQ dots with a variety of emission wavelengths enables multicolor 3D labeling that can be used for protein mapping at super-resolutions in myofibrils and other samples.

1097-Plat
Structural Changes in the Myosin Motors During Activation of Skeletal Muscle
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Structural changes in the myosin motors during the transition from the resting state to the plateau of isometric contraction were investigated by X-ray interference from single fibers of frog skeletal muscle. Isolated intact fibers (2.1μm sarcomere length, 4°C) were mounted vertically at beamline ID2 of the ESRF synchrotron (Grenoble, France) between a loudspeaker motor and a capacitance force transducer. 2D diffraction patterns were collected on a CCD detector 10 m from the preparation with 5 ms time resolution. During the development of the isometric tetanus, the intensity of the M3 reflection, originating from the axial repeat of the myosin motors, first decreases to 30% (at 50 ms) of its resting value, then increases to a steady value 70% of that at rest. The M3 reflection has a major peak with spacing 14.34 nm at rest and two peaks with mean spacing 14.57 nm at the tetanus plateau (Linari *et al.*, *Proc. Natl. Acad. Sci. USA* 97:7226, 2000). The changes in the fine structure of the M3 reflection during activation were best fit by a structural model in which (1) all thick filaments have the same mean spacing at a given time during activation (2) the number of active motors increases in proportion to the isometric force (Brunello *et al.*, *J. Physiol.* 577:971 2006), (3) the conformation of the active motors is independent of the level of force and strain in the thick filament.
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1098-Plat
Measurement Of ATPase Activity During Ramped Stretches In Contracting Skeletal Muscle Fibers Of The Rabbit
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Skeletal muscle force response to small amplitude and low velocity ramp stretches is biphasic. An initial fast increase in force, where myosin heads are forcibly detached is followed by a slower increase in force with net re-attachment of myosin heads to actin. The average ATPase rate is very low during stretch (Curtin & Davies,1973), but due to lack of time resolution the two phases to match force changes have never been resolved. We therefore examined and modeled tension and ATPase responses to ramp stretches (5% and 1% of fiber length, Lo) at low velocities (0.1 and 0.5 Lo/s) in permeabilised fiber bundles of rabbit psoas at 12 and 20°C. We show that ATPase activity drops to near zero during the initial fast phase of the stretch and increases slightly but still remains lower than isometric during the second part of the stretch phase, returning to normal post-stretch. The response was not as marked at 12 as at 20°C, although ATPase rate was still reduced in both the fast initial and slow secondary phase of the force response. During the initial phase the myosin heads are forcibly removed from actin, the cross-bridge cycle is not complete and release of hydrolysis products is interrupted. In the second phase, myosin heads re-attach whilst the muscle is still lengthened and the cross-bridge cycle is truncated for a fraction of attached heads, leading to slower Pi release than during isometric conditions. These effects are less marked at 12 than at 20°C because the fraction of strongly bound cross-bridges is reduced at the lower temperature.
Curtin, N.A. & Davies, R.E. (1973). Cold Spring Harbor Symp. on Q. Biol., 37, 619-626.

1099-Plat
Disrupting Myosin Relay-Converter Domain Communication Impairs Drosophila Muscle Mechanical Performance and Flight Ability
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