**3170-Pos Board B217**

**Modeling Muscle With A Continuum Approach, New Insights Into An Old Problem**

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Muscle contraction has long been modeled using partial differential equations (PDEs) to describe the chemical states of myosin as a function of time and strain. These models typically assume constant shortening rate and sparse myosin binding sites on actin. However, oscillatory shortening occurs in Monte-Carlo simulations of small ensembles of myosin (N=150) with fixed near-stall load and continuous binding sites (e.g. Duke 2000). Are these oscillatory solutions physiologically important? Should we re-examine the convenient assumptions of constant shortening rate and sparse binding? If so, then we should find oscillations in realistic PDE muscle models without these assumptions. Here, we develop a realistic muscle model. Using a soft-spring approximation, where we assume that external force on myosin induces a deformation primarily along a single degree of freedom, we develop relationships between rate constants and strain. These rate functions may be defined with a few parameters. The parameters not known from biochemical and biophysical studies may be determined by an optimization-of-fit to data. This approach generalizes previous expressions (e.g. Duke 1999, Smith and Geeves 1995). We then develop two models: 1) a PDE model with discrete binding sites and fixed load. This model shows near-stall oscillations only with finite N. 2) an integro-PDE model with continuous binding sites (after Hoppensteadt and Peskin 1992) and fixed load. This model exhibits oscillations that decay to a uniform steady-state. We introduce a reduced model to explain the N-dependence of these large load-oscillations and show that they are a result of small (N~O(102)) ensemble size. Thus, we argue that the continuum modeling approach should not be rejected based on large-load oscillations or continuous binding sites.

**3171-Pos Board B218**

**Short-range Mechanical Properties Simulated With A Mathematical Model Incorporating Multiple Half-sarcomeres**

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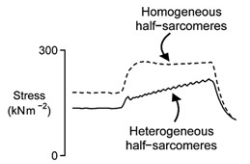
When an activated skeletal muscle fiber is stretched, force rises rapidly until the muscle reaches its 'elastic limit'. This 'short-range' response probably reflects the effects of interfilamentary movement on the dynamic behavior of cycling cross-bridges. Most mathematical models of the response predict that the cross-bridge force will plateau during the latter stages of the stretch. There are however some experimental preparations (including rabbit psoas fibers) in which force continues to rise at a gradual rate beyond the elastic limit. One explanation for this behavior is that titin filaments have calcium dependent mechanical properties. Another possibility is that the slow increase in tension beyond the elastic limit reflects dynamic interactions between inhomogeneous half-sarcomeres. A mathematical model of 40 half-sarcomeres arranged in series was developed to test this hypothesis. The solid line in the figure shows the simulated response when half-sarcomeres at the ends of the model had fewer cross-bridges than half-sarcomeres in the middle. The dashed line shows the simulated response when all the half-sarcomeres were identical. These simulations suggest that the gradual rise in tension beyond the elastic limit observed in some preparations may reflect interactions between inhomogeneous half-sarcomeres.




**3172-Pos Board B219**

**A Simple Two-state Model For Auto-oscillation Of Sarcomeres (SPOC)**

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The contractile system of striated muscle usually takes either contraction or relaxation state, which is regulated by the concentration of free Ca2+. On the other hand, we found that under the conditions intermediate between contraction and relaxation, the auto-oscillation of sarcomeres (named SPOC) occurs (Okamura, N. and Ishiwata, S., 1988. J. Muscle Res. Cell Motil. 9, 111-119). During SPOC, each sarcomere repeats the cycle of slow shortening and rapid lengthening periodically under constant micromolar [Ca2+] (Ca-SPOC) or in the presence of high concentration of MgADP and the absence of Ca2+ (ADP-SPOC). Many experimental results on the characteristics (e.g., period and amplitude of oscillation of sarcomere length) of SPOC have been obtained under various conditions (various concentrations of free Ca2+, MgADP and inorganic phosphate, pH and temperature). However, the molecular mechanism of SPOC is not yet clearly understood.

Recently, we observed that the width of sarcomeres, which corresponds to the lattice spacing between the thick and thin filaments, also oscillates during SPOC. It was found that the subtle change (less than nm) in the lattice spacing is responsible for the SPOC to occur. Based on these experimental findings, we constructed a simple two-state model to explain the SPOC phenomena, where the lateral force balance in addition to conventional longitudinal force balance was newly taken into account.

**3173-Pos Board B220**

**On the physics of muscle contraction Force - velocity**

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Whichever energy source is chosen as an engine, its force will decrease with increasing velocity. This is connected with a limited power of any engine. Thus, Hill's formula is a mere sequence of the law of energy conservation.

To derive a mathematical dependence ''force-velocity'', all the means of consumption of fuel energy should be determined - in our case, the energy of the ATP hydrolyze. Moreover, the conformation energy of the crossbridges attached serves as the force source as well.

We state that part of the energy release transforms into the energy of oscillations of myosin proteins; the other part goes into thermal energy of the sarcoplasmic solution. Interaction of the oscillating myosin system with the sarcoplasmic solution controls the process of force generation by a muscle. It is just this interaction that leads to the temperature dependence of force.

The presentation is devoted to constructing a theory based on these simple considerations.

A constructive critic is especially wanted.

**3174-Pos Board B221**

**The Fluorescence Lifetime of a Single Actin-bound Fluorophore During Contraction of Skeletal Muscle**

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During interaction of actin with myosin, cross-bridges impart cyclical impulses to thin filaments. A cross-bridge spends part of cycle time strongly attached to actin ($t_s$) during which it generates force, and remaining time ($t_d$) detached from thin filaments. The environment of a binding site on actin is different when a cross-bridge is attached and detached from thin filaments. Here we report, for the first time, measurements of the environment of a single actin binding site in rigor, relaxed and during isometric contraction of skeletal muscle. The environment was monitored by tracking the fluorescent lifetime ($\tau$) of single Alexa488-phalloidin molecule bound to actin. The fluorescent lifetime is the averaged rate of decay of fluorescent species from the excited state. It depends on a variety of environmental factors. Lifetime of a single phalloidin molecule located at the center of the Overlap-band was measured every 50 msec during 60 sec of rigor, relaxation and contraction of muscle. The lifetime of rigor muscle was large when a cross-bridge was bound to actin, low when it was dissociated from it and intermediate during contraction. The ''duty cycle'' of a cross-bridge ($\Psi$) - defined as the fraction of the total cross-bridge cycle that myosin spends attached to actin in a force generating state [$\Psi = t_s/(t_s+t_d)$] was calculated from lifetime as 60%.

**3175-Pos Board B222**

**Modelling X-ray Diffraction From The Myosin Superlattice Of Vertebrate Muscle**

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Muscular force is generated by molecular interactions between the contractile proteins actin and myosin. The myosin filaments in the sarcomere of vertebrate muscle pack on a triangular array into which the actin filaments are interdigitated. High resolution studies of the actin-myosin interactions are performed by x-ray fiber diffraction analysis of whole muscle fibers. In most vertebrate muscles however, the myosin filaments pack in a so-called ''superlattice'' arrangement that involves a semi-random distribution of two filament

rotations. The unknown effects of this disorder on diffraction by muscle fibres have so far prevented a rigorous analysis of x-ray fiber diffraction patterns in terms of the structure of this complex system. We report a quantitative model of the disorder and its incorporation into calculations of x-ray fiber diffraction patterns from model structures. This allows rapid calculation of the diffraction and does not involve numerical averaging over the disorder. Calculations show that the disorder modulates the Bragg reflections in diffraction patterns and introduces diffuse diffraction. The results of this analysis will allow the effects of the disorder to be included in muscle structure refinement programs, allowing more accurate structure determination from x-ray fiber diffraction data.

**3176-Pos Board B223**

**The Intensity Of The 2.7nm Reflection As A Constraint For Models Of Myosin Docking To Actin**

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Previous workers have proposed high resolution models for the docking of the myosin heads on actin on the basis of combined crystallographic and electron microscopy data (Mendelson and Morris, 1997 *PNAS* **94**:8533; Holmes *et al.* 2003 *Nature* **425**:423). We have used data from small angle X-ray fiber diffraction from living muscle to check the predictions of these models. Whole *sartorius* muscles from *Rana pipiens* were mounted in a chamber containing Ringer's solution at 10°C and at rest length at the BioCAT beamline (18 ID, Advanced Photon Source, Argonne, IL-U.S.A.). The muscles were activated by electrical stimulation and the force was recorded with a muscle lever system type 300B (Aurora Scientific). X-ray patterns were collected with 1s total exposures at rest and during isometric contraction out to 0.5 $nm^{-1}$ in reciprocal space, as the higher angle reflections are expected to be more sensitive to the arrangement of myosin heads on actin. We observed that during isometric contraction the meridional reflection originating from the 2.73nm repeat of the actin monomers along the actin filament increases its intensity by a factor $2.1 \pm 0.2$ relative to rest. Among the models tested, Holmes *et al.* fits the data when the actin filament is decorated with 30-40% the total available myosin heads, a fraction similar to that estimated with fast single fiber mechanics by Piazzesi *et al.* (2007, *Cell* **131**:784). However, when the mismatch between the periodicities of actin and myosin filaments is taken into account, none of the models can reproduce the fiber diffraction data. We suggest that the fiber diffraction data should be used as a further constraint on new high resolution models for the docking of the myosin heads on actin.

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**3177-Pos Board B224**

**New X-ray Data about Myosin-binding Protein C in Frog Muscle**

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Frog striated muscle gives many meridional X-ray reflections at spacings greater than 400Å which are still incompletely understood. Some come from C-protein, as discovered by Offer (CSH Symp.37, 83-97, 1972) and by Rome (ibid,331-339). Others may come from a ''forbidden'' first order myosin meridional reflection, as discussed by Malinchik and Lednev (JMRCM.13, 406-419, 1992). In both cases the reflections will be split by interference fringes from the two half A -bands. Squire has suggested that the apparent C-protein repeat of ~ 435Å (rather than 429Å) in relaxed muscle may be due to interaction with actin (JMB.331, 713-724, 2003).

We have studied these reflections at high resolution on the BioCAT beam line at the Argonne National Lab., in relaxed and contracting muscle. In resting muscle, two main peaks occur in the relevant region, at ~419Å and ~442Å, the latter being about 4 times more intense than the former, indicating an underlying repeat of ~437Å and an apparent interference distance of ~8200Å (and an actual one of ~7100Å). In contracting muscle, the pattern is very much weaker, and the corresponding spacings are 412Å, 447Å (i.e. a wider doublet), 440Å underlying spacing, and 5300Å apparent interference distance. Part of the change may be ascribed to a weakening of the presumed contribution from the myosin ''forbidden'' meridional reflection, but the apparent interference distance would now indicate an actual distance of ~4460Å, short compared to the expected value (~5900Å) for C-protein ; the long C repeat spacing (440Å) is also notable.

At lower angles still, the observed reflections index as the higher even orders of the sarcomere repeat, as reported by Bordas and colleagues (J. Cell. Biol.105, 1311-1318, 1987), and which shorten in contraction. But why only even orders appear is still an intriguing puzzle.

**3178-Pos Board B225**

**An Automated Apparatus for Isometric Force Analysis of Skinned Muscle Fibers**

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Skinned muscle fibers provide a powerful means to assess the functional effects of compounds that modulate the sarcomere. The main drawback of this preparation as an assay system is its low-throughput nature. As part of an effort to optimize small molecule activators of the skeletal sarcomere for therapeutic applications in conditions where muscle weakness is a feature, an automated system was designed that can simultaneously run multiple types of isometric force assays. Six identical units, controlled through a single software interface, run a variety of assay protocols. Each unit independently measures the force of a single suspended fiber as it is submerged into various solutions in a temperature controlled block. Assay protocols are unique for each tissue type and desired measurement. Fiber quality is automatically assessed by switching between fully contracting and fully relaxing pCa solutions. If sufficiently robust, fibers are tested by indexing between solutions of varying pCa or compound concentration. In each new solution, the software monitors the rate of force generation and when the fiber has reached a force plateau, automatically moves to the next solution. Control pCa profiles of rabbit psoas fibers measured over a year and a half period show typical variation of $< 0.1$ pCa unit from historic values. This capability has allowed characterization of several hundred compounds aiding with the selection of a troponin activator as a development candidate for diseases characterized by muscle weakness.

**3179-Pos Board B226**

**A White Noise Approach To System Analysis In Demembranated Muscle Mechanics**

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Measuring the force response to sinusoidal length perturbations in muscle enables calculating the viscoelastic properties of the tissue over a wide range of frequencies. Coupling these empirical results with complementary mathematical and computational models describes the kinetics of force-generating actomyosin cross-bridges. This sinusoidal analysis requires system linearity, a constraint confining length stimuli to very small amplitudes in demembranated (skinned) muscle preparations because larger length perturbations produce a non-linear force response. Therefore, it becomes difficult to examine cross-bridge cycling kinetics during length transients that are comparable with sarcomeric strains experienced during contraction in living muscles. Here we introduce a white noise method of system analysis that facilitates extracting the linear and non-linear components of the system response. Building upon Wiener theory, this method estimates the system response to a band-limited Gaussian white noise length stimulus through cross-correlation techniques (Lee-Schetzen approach). To examine and develop this approach, we computer simulated the response of a predefined system consisting of both linear and non-linear components and were able to estimate the expected linear response of the system. These simulations demonstrate the powerful utility of this technique to separate the linear and non-linear system responses in both the time or frequency domains. We also examined the experimental applicability of these methods using small strips of skinned muscle tissue, from which we estimated the linear and non-linear components of the system response in calcium-activated muscle. This linear component is consistent with the linear system response calculated from comparable measurements using sinusoidal length perturbation analysis. These computational and experimental methods provide a platform for characterizing cross-bridge cycling behavior, and permits distinguishing between linear and non-linear components of the complicated force responses following length transients associated with normal muscle contraction.

**3180-Pos Board B227**

**Repetitive Contractions at Short and Long Lengths: Do Not Subtract Passive Force!**

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Several reports have compared the consequence of repetitive contractions at long and short lengths, with the goal of gaining an understanding of factors causing muscle fatigue: metabolic vs ion distribution. This is traditionally done calculating active force as peak force - passive force. Alternatively, it has recently been shown that during contraction of whole muscle, fascicle length shortens, and it would be more appropriate to subtract the passive force associated with the fascicle length at the peak of the contraction. These two approaches will give different results, for contractions at long length. Contractions of the rat medial gastrocnemius muscle were obtained at 0.3Hz (trains