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.

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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 ± 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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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.

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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.

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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.

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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