ESRF	**Experiment title:** Structural dynamics of muscle contraction: a combined mechanical and time-resolved X-ray diffraction study on single muscle fibres	**Experiment number**: LS-1262
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Report: The experiments reported here (LS-1262, April 99) relate to points A and B (first and second allocation period) of the proposal for a Long Term Project (March 1999).

According to recent crystallographic studies (Holmes, *Curr. Biol.* **7**, R112, 1997) force generation in the actin-myosin cross-bridge (the working stroke) is due to tilting of the light chain binding domain of the myosin head, the lever arm, about a fulcrum (res. 690-710) in the catalytic domain attached to actin.

During LS 1013 we determined for the first time in the same single muscle fibre the interference fine-structure of the meridional myosin-based reflections at the plateau of an isometric tetanus, T_0 (myosin heads at the beginning of the working stroke) and in rigor (end of the working stroke). The difference in interference splitting of the M3 reflection (the intense third order myosin meridional reflection due to the ~14.5 nm axial repeat of myosin heads) supported the idea that at T_0 the lever arm is axially tilted by ~30º from the nucleotide-free conformation of Rayment *et. al*. (*Science* **261,** 58, 1993), which is generally assumed to correspond to rigor. Thus the axial movement between T_0 and rigor is ~5 nm, in agreement with the structural model used to simulate M3 intensity changes accompanying synchronous movements of the head elicited by step perturbations in length (Dobbie *et al*., *Nature* **396**, 383, 1998). However the interference fine-structure of the M3 is also sensitive to the strain in the myosin heads, which was much smaller in our previous rigor measurements than at T_0. We therefore measured the interference fine-structure in rigor at different steady forces to characterise the effect of strain. This also provides a sensitive test of the myosin filament model.

Experimental protocol: Single fibres from the tibialis anterior muscle of *Rana temporaria* were mounted horizontally in a trough containing Ringer solution at 4 °C, between a force transducer and a loudspeaker coil motor. In rigor experiments, low tension rigor (LT) was induced by MgATP depletion (Linari *et al. Biophys. J.* **74**, 2459, 1998) after chemical demembranation (Triton X-100, 0.5 % in relaxing solution, 20

min at room temperature). Two mica windows carrying the electrodes were moved as close as possible (~ 600 µm) to reduce the X-ray path through the solution. The trough was mounted vertically with the transducer at the top and the motor at the bottom. To maximise spatial resolution, X-ray patterns (20 s exposure) were collected on a storage phosphor image plate detector (IP, A3 size) 10 m from the fibre. High tension in rigor (HT) was obtained by slowly stretching the rigor fibre (0.02 µm/s per half-sarcomere, amplitude ~10 nm per half- sarcomere; see Linari et al., 1998). Because of the slow extraction/insertion of the IP, data were usually collected for only one mechanical condition from each fibre. To spread the radiation damage, the fibre and the stage were vertically oscillated during the exposure. IPs were scanned with 100 µm spatial resolution (Molecular Dynamics). Data analysis was performed using the program HV written by Dr A. Stewart and Peakfit software package (Jandel Scientific).

Results: When the rigor fiber is slowly stretched to attain a steady tension of about 0.5 T_0, the intensity of M3 reflection increases by about 70 % with respect to rigor LT and the splitting of the reflection changes so that there is a main peak at spacing 14.46 nm and two satellites peaks of amplitude ~ 10-15 % that of the main peak (Fig. 1). The splitting of the M3 reflection was reproduced by a structural model of the myosin filament, assuming that (1) in rigor LT the heads have the same conformation as the nucleotide free crystallographic structure (Rayment et al., 1993) and spacing 14.44 nm; (2) the M3 reflection is sampled by the interference function generated by two arrays of 49 heads with opposite polarity in the two halves of the myosin filament; (3) the length of the bare zone in rigor LT is 1% smaller than previously assumed for isometric contraction, in proportion to the reduction in axial spacing of the M3 reflection. When the model is applied to the intensity profile in rigor at 0.5 T_0 (HT), both the increase in the integrated intensity of the M3 and the changes in its sub-peak composition are reproduced if the light chain region is bent away from the barbed end of the actin filament by 0.7 nm (corresponding to myosin head compliance in rigor; Linari *et al.* 1998) and also back-tilted by 13°. These results show that the interference effect allows determination of the polarity of myosin head movements in the sub-nm range.

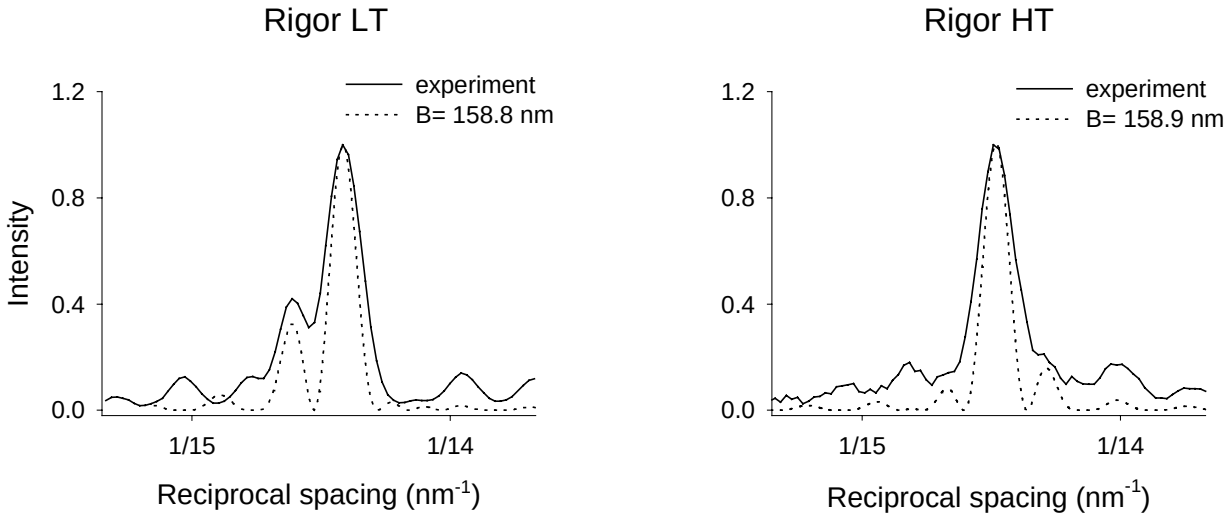

Fig. 1. Superimposed experimental (continuous line) and simulated (dotted line) intensity at the level of the M3 reflection in rigor at zero tension (LT, five fibres, 385 s total exposure) and in rigor at 0.5 T_0 (HT, three fibres, 60 s total exposure). To compare the profiles, intensity is made relative to the height of the main peak of the reflection. For the best fit of HT rigor M3 spacing is increased from 14.44 nm (LT rigor) to 14.45 nm, according to the increase in filament length expected from myosin compliance (0.12 % T_0^{-1}, Dobbie *et al.* 1998). The length of the bare zone (B) is changed according to the change in M3 spacing.