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-1013
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Report: The experiments reported here (LS-1013, sept 98) were planned in the proposal for a Long Term Project at point B "Changes in conformation of myosin heads during the ATPase cycle", second allocation period. A paper is in preparation.

According to the most recent crystallographic studies (Holmes, *Curr. Biol.* **7**, R112, 1997; Dominguez *et al.*, *Cell* **94**, 559, 1998) 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. To define the polarity and the extent of tilting of the lever arm during the working stroke, the interference fine-structure of the meridional myosin-based reflections at the plateau of isometric tetanus (myosin heads at the beginning of the working stroke) was compared to that in rigor (heads at the end of the working stroke). Because of the asymmetry in the disposition of myosin heads in the two halves of the myosin filament, the interference effect, generated by the arrays of heads in the two halves of the filament, changes as a consequence of the conformational change accompanying the working stroke. An unprecedented spatial resolution in the two-dimensional patterns was attained as follows: *i*, fibres were mounted vertically in a specially devised trough, so that the meridional axis was parallel to the smallest dimension of the X-ray beam; *ii*, patterns were collected on a storage phosphor image plate detector (IP, A3 size) placed at 10 m from the specimen.

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, rigor 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 apart) to reduce the X-ray path through the solution. The trough was mounted vertically on the beam line with the

transducer at the top and the motor at the bottom. The solution was maintained in the trough by means of a perspex cover sealed with silicone grease. X-ray exposure was limited to the period of data acquisition by a fast shutter (switch time ~ 5.4 ms). Data during isometric contraction were obtained by adding frames at the plateau of 2 s tetani. The plate carrying the fibre was shifted vertically by 400 µm after each exposure to spread and minimise the radiation damage. 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: Both at the plateau of the isometric tetanus and in rigor M3 reflection was split in two sub-peaks whose separation was ~ 1000 nm as expected from interference between the two halves of the sarcomere, but the relative amplitudes of the peaks and their positions (estimated with a multiple gaussian fit) were markedly different (Fig. 1). At the isometric tetanus plateau (4 fibres) the two peaks of the M3 reflection are of comparable size: the intensity of the high angle peak at 14.47 nm is 84 ± 6 % of that of the low angle peak at 14.67 nm. The centre of mass of the M3 reflection in the tetanus is at a spacing of 14.57 nm, 1.6% larger than the centre of mass of M3 at rest. In rigor (5 fibres) there is a main peak at 14.40 nm and a satellite (intensity 28.6 ± 1.6 %) at 14.60 nm; the centre of mass is at 14.44 nm, only 0.7 % larger than that at rest. The analysis of the second order of M3 reflection (M6) shows that: *i*, at the tetanus plateau the M6 reflection is split in two peaks with the intensity of the high angle peak about ½ of that of the low angle peak; *ii*, in rigor there is only one peak at 7.23 nm. The changes in the interference fine-structure of the myosin based meridional reflections M3 and M6 have been simulated by using the crystallographic co-ordinates provided by Rayment *et al*. (*Science* **261,** 58, 1993) for nucleotide-free (rigor) S1 portion of the myosin head. The M3 and M6 Fourier components of the myosin heads were calculated for the rigor conformation at spacing 14.44 nm and for the isometric tetanus (T_0) conformation at spacing 14.57 nm, assuming as in Dobbie *et al*. (*Nature* **396**, 383, 1998) that at T_0 the lever arm is tilted by 30 º up from the rigor conformation of Rayment *et. al*. The interference function sampling the reflection is generated by two arrays of 49 heads with opposed polarity in the two halves of the myosin filament. The only free parameters to adjust in the simulation are the length of the bare zone (B) and the dispersion of conformations of attached heads (D) expected from the mismatch between the periodicity of the myosin and actin filaments. These results show that the interference effect can be used to determine sub-nanometre changes in the structure of the myosin motor associated with the chemo-mechanical energy transduction.

Fig. 1. Integrated intensity along the meridian at the plateau of an isometric tetanus and in rigor from a single muscle fibre.