ESRF	**Experiment title:** Experimental determination at high resolution of the meridional phases in X-ray diffraction patterns from live contracting muscles.	**Experiment number**: LS-1252
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Report: X-ray diffraction patterns from resting, isometrically and isotonically contracting muscles were recorded in a range from ca.1200 to 2.5 nm, with enough resolution to resolve the sampling by an interference distance of ca. 850 nm of the meridional myosin orders. These orders come from the axial disposition of the myosin heads and the interference occurs between myosin diffraction units on either side of the M line. Part of this work (Biophys. J.; in press) has shown that: i) few, if any, heads form an acto-myosin (AM) complex during isotonic shortening; ii) ca. 50% form a stereo-specific AM complex during isometric contraction, and; iii) isometric contraction occurs against a thin filament compliance of at least 0.646 ± 0.046 % nm/P_o, where P_o is the value of maximum isometric tension.

Phasing of the meridional diffraction diagrams, as outlined in the LS-1252 application, is partly completed. For resting and isometrically contracting muscles phasing has been achieved to a resolution of ca. 2.9 nm. So far, the data from isotonically contracting muscles is good enough up to a resolution of ca. 4.9 nm. By way of illustrating the data quality, Fig. 1 shows the 6^{th} order myosin meridional reflection (6M) at P_o (circles). The 6M is a cluster of

peaks due to interference effects, and similar effects occur in all other diffraction orders. These clusters of peaks we argued could be exploited to obtain phases. The unlabelled line in Fig.1 shows the theoretical fit from which the phase was derived. Fig. 2 shows the experimentally determined electron density map (circles) at P_o at a resolution of 2.9 nm. The continuous line, superimposing on the electron density map is the only mass projection of a pair of myosin heads [1] that fits the data. The derived disposition of one pair of heads is shown in the inset (muscle axis assumed to be vertical). The significance of this result is that the configuration of each head is different, with one of them in a perpendicular orientation to the thin filament, whilst the other essentially retains the configuration typical of heads in resting or isotonically contracting muscles (data not shown here). Two different forms of interaction between actin and myosin are needed to explain mechanical studies [2] and have been predicted from molecular dynamics calculations and energy considerations [3].

[1] Rayment , Rypniewski , Schmidt-Bäse , Smith, Tomchick, Benning, Winklemann, Wesenberg and Holden. 1993, Science, **261**, 50-58.

[2] Huxley and Simmons. 1971, Nature, **233**, 533-538.

[3] Diaz-Baños, Bordas, Lowy and Svensson. 1996, Biophys. J. **71**, 576 –589.