ESRF	**Experiment title:** Molecular mechanism of muscle contraction in demembranated fibres	**Experiment number**: SC-3473
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Report:

The aim of this project was to develop an improved permeabilised cell preparation that would allow a new generation of synchrotron X-ray studies of molecular mechanisms of contraction and regulation in skeletal muscle. In fact skinned muscle fibres (isolated muscle cells in which the surface membrane has been removed or permeabilised) allow the concentrations of small molecules bathing the contractile filaments to be controlled, while preserving the fundamental lattice of the filaments, and allowing both mechanical and structural measurements on the filament array. Our recent results at ESRF (SC-2989) showed that the myosin filament structure of skinned muscle fibres from rabbit psoas in relaxing condition at physiological temperature is different from the structure of intact muscle fibres at rest. Thus a first goal pursued during this visit (SC-3473) was to determine the optimal solution conditions for the permeabilised cells to best match the native physiological structure by (a) changing the ionic strength of the solution bathing the myofilaments in the range 150 to 230 mM, and (b) recovering the native interfilament spacing by addition of an osmotic agent (long-chain polymer dextran T500). Once these conditions were defined, we determined the changes in the x-ray pattern upon fibre activation at different levels of calcium concentration. 20 ms X-ray frames were collected using a CCD FReLoN detector (active area 10x10 cm^2, pixel size 52μm, possibility of binning 8x in direction perpendicular to the fibre axis) and a camera length either 6m or 3m. A small beam (120 x 120 μm^2, obtained via the slits) and the high brightness at ID02 allowed us to have higher spatial resolution compared with previous experiments (SC-2989) at both camera lengths so that now x-ray interference between the two halves of the sarcomere can be recorded from horizontally mounted demembranated fibres.

Fibre preparation and mechanical protocol. Demembranated bundles of fibres from rabbit psoas muscles were prepared in Florence and stored and transported to the ESRF at -20°C in relaxing solution containing 50% glycerol. Before the experiments bundles of 3-5 fibres 5-6mm long were isolated, their extremities clamped with aluminium T-clips and horizontally mounted in a drop of relaxing solution between the lever of the motor and the force transducer. When necessary 5% w/v dextran T500 was added to the solutions to change fibre interfilament spacing. Cycles of contraction-relaxation with preservation of sarcomere structure were obtained by using the solution exchange system combined with temperature jump (Linari *et al.*, Biophys. J. 92:2476, 2007). The thermo-regulated trough has a Z movement that allowed the plate carrying the solution drops to be lowered so that X-ray measurements could be made with the fibre in an air cavity in

the centre of a temperature controlled aluminium block.

Results. In the conditions of the present experiments, no significant changes were recorded in the x-ray diffraction pattern varying the ionic strength (via [EGTA]) of the relaxing solution in the range 150-230 mM. At the tested temperature of 12°C, addition to the relaxing solution of 5% w/v of the osmotic agent dextran T500, known to induce the recovery of the intact fibre interfilament spacing, causes (i) the increase of the intensity of the first myosin layer line and of all the myosin-based meridional reflections (in particular of the so-called forbidden reflections) and (ii) the decrease of the spacing of the myosin-based meridional reflections. In intact amphibian muscle cells removal of the resting Myosin Binding Protein-C (MyBP-C) links between the myosin and actin filaments induces the decrease of the intensity of the forbidden meridional reflections and increase of the spacings of the myosin-based meridional reflections indicating the loss of the triplet perturbation of the axial ordering of the crowns of myosin heads on the myosin filament (Reconditi *et al.* 2014). Thus the above results in demembranated fibres suggest that the recovery of the interfilament spacing induces the recovery of the triplet perturbation of the resting structure characteristic of the amphibian muscle, likely because of the recovery of the MyBP-C "links". Force-calcium titration in the presence of 5% dextran (Fig. 1A) exhibits an increased calcium sensitivity with respect to control in the absence of dextran, indicating a more efficient activation when interfilament spacing is recovered. The M3 meridional reflection from the axial repeat of the myosin heads was sampled by X-ray interference between half-sarcomeres (Fig 1B). In relaxed fibres at 12°C in the presence of 5% dextran, the M3 reflection had a major peak at 14.34±0.01 nm, with small satellite peaks on either side, as in resting intact fibres from the frog (Fig. 1C, LM3, ratio of the intensity of the low angle peak and total M3 intensity = 0.07±0.12). Activation at different [Ca^{2+}] redistributes the intensity of the three peaks of the M3 reflection. From pCa 6.5 (or force 0.3 the force at full activation T_0), the M3 reflection is split only in two peaks at 14.6 nm and 14.4 nm and the fine structure and the spacing of the reflection remain nearly constant (*L*M3 = 0.61±0.03, Fig 1C, and *S*M3 =14.54±0.02 nm, Fig 1D), while the intensity of the reflection (related to the number of attached motors, not shown) increases linearly with force or [Ca^{2+}]. These results indicate that at pCa 6.5 or force 0.3 T_0 the thick filament and the myosin heads switch completely to the active structure.

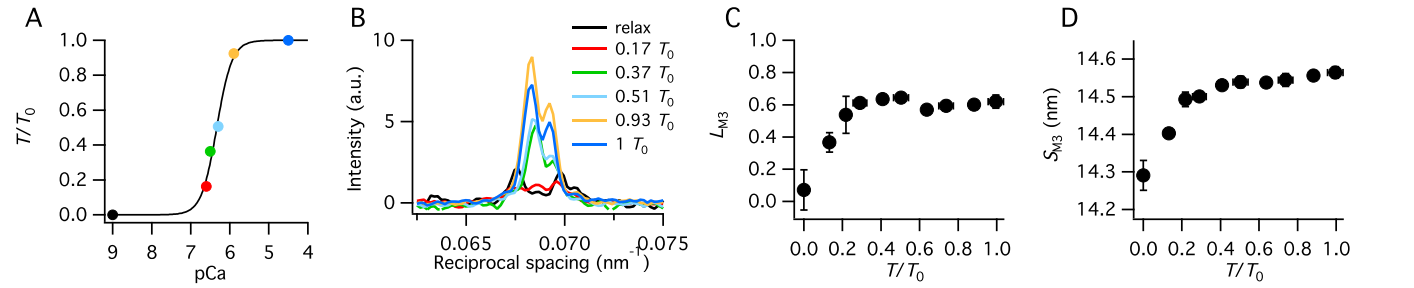

Fig. 1. M3 reflection of fibres activated with different levels of calcium concentration.

Publication from ESRF in the last 18 months.

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Abstracts

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