ESRF	**Experiment title:** Control of contraction in slow-twitch skeletal muscle	**Experiment number**: LS3080
Beamline: ID02	**Date of experiment**: from: 31/05/2022 to: 3/06/2022	**Date of report**:
Shifts: 9	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

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Report:

Muscle contraction is driven by sliding between thick (myosin-containing) and thin (actin-containing) filaments, and triggered by a transient increase in calcium ion concentration in the muscle cells. The calcium ions bind to the thin filaments, causing a structural change that permits binding of the myosin motor domains to actin, providing a start signal for contraction. The strength, speed and metabolic cost of contraction are controlled by a second set of regulatory structural changes in the thick filaments in which the myosin motors are released from the helical folded conformation on the surface of the thick filaments that made them unavailable for binding actin in resting muscle (Irving M 2017 *Biophys J* 113: 2579). Release of the motors from the helical folded state is triggered mechanically, by the strain in the thick filament (Linari M *et al*. 2015. *Nature* 528:276; Hill C *et al* 2022 *J Physiol* 600.17:3983). This intrinsic mechano-sensing mechanism couples the number of active motors to the external load on the muscle, maximizing the efficiency of contraction, but its molecular structural basis remains unknown.

In LS3080 we exploited the unique capability of ID02 with the high time- and spatial-resolution of the Eiger2-4M detector to investigate this mechanism in intact mammalian skeletal muscle. With the detector at 31m from the muscle we were able to clearly resolve the first-order reflection from the sarcomere repeat for the first time in intact muscle, allowing sarcomere length to be determined precisely with 2-ms time resolution. We mounted EDL muscles of the mouse at an initial sarcomere length of 2.8 μm at 27°C and stimulated them at 130Hz for 120 ms. At 60 ms, when developed force was nearly maximal, we imposed sarcomere shortening of 20% in 15ms to bring force transiently to zero, before it was allowed to redevelop at the shorter sarcomere length, then mechanically relax at the end of stimulation. This protocol is designed to determine the effect of force on the activation states of the thin and thick filaments when calcium activation is maximal.

We then recorded diffraction patterns at a sample-to-detector distance of 3.2 m in 2 ms time frames throughout this protocol, ending 80 ms after the end of stimulation, in order to measure changes in the equatorial reflections, myosin-based layer lines, myosin-based meridional reflections (in particular the interference fine-structure of the M3 reflection from the axial repeat of the myosin motors that also reveals the region of the thick filament in which motors are activated), the spacing of the M6 reflection that mediates mechano-sensing in the thick filament backbone, troponin-based meridional reflections and the second actin-based layer line that reports the azimuthal position of tropomyosin and the activation state of the thin filament. We recorded a total of 87 repeats of this protocol in five muscles, providing very good signal:noise of all these reflections at 2ms time resolution, plus the necessary control runs.

Analysis of the large quantity of data collected in LS3080 is still ongoing, but we already obtained unprecendented resolution of the sarcomere length changes, lattice spacing changes, equatorial intensities and intensity, spacing and interference fine structure of the meridional M3 and M6 reflections. These signals have not been recorded previously with this type of protocol in mammalian muscle, and both the signal:noise and time resolution are much better than in published data in amphibian muscle. One notable result so far is the transient appearance of a new X-ray reflection corresponding to a periodicity of 14.8 nm during unloaded shortening at full calcium activation, accompanied by an anomalous decrease in the spacing of the M3 reflection while that of the M6 reflection is increasing. These phenomena have only been seen previously at the start of stimulation when thin filament activation is incomplete (Hill C *et al*. 2021 *eLife* 10:e68211; Hill C *et al* 2022 *J Physiol* 600.17:3983), and their presence during unloaded shortening at maximal calcium activation puts an important constraint on hypotheses about the mechanism of dual filament regulation.