ESRF	**Experiment title:** Control of Contraction in Skeletal Muscle by Thick Filament Mechano-sensing	**Experiment number**: LS3041
Beamline: ID02	**Date of experiment**: from: 10/11/2021 to: 12/11/2021	**Date of report**:
Shifts: 6	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

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Report:

The aim of this project was to use the upgraded ID02 beamline with the Eiger 2-4M detector to determine the regulatory structural changes in the myosin-containing thick filaments following single action potential stimulation of isolated mammalian muscles in physiological conditions, and their dependence on muscle load. The broader significance of that aim is that no previous study has investigated thick filament mechano-sensing in mammalian muscle during physiological activation by a single action potential, and the results could lay the foundations of a structural and functional framework for testing small molecules as potential therapeutics for muscle weakness in man.

We applied for 18 shifts which would have allowed us to complete the three related protocols described in the application that, combined, should have provided the mechanistic understanding of thick filament regulation in mammalian skeletal muscle, constituting that framework according to the above aims. However we were awarded only 6 shifts, which would have allowed us to complete only one of the three protocols under ideal

circumstances. Unfortunately nearly all of the first three shifts were lost because no useful beam was available at ESRF as a result of an RF problem. Since almost the whole of one shift is required to optimise the beam conditions and instrumentation for the mouse EDL muscles, which we had not used before at ESRF, only two shifts were available for the proposed experiments, making it unfeasible to achieve the statistical power required for even one of the protocols planned in the application.

To make the best use of the time available, we therefore switched to a simpler experimental protocol in which longer periods of data acquisition could be employed to increase the signal:noise in each muscle, so that fewer muscles would be necessary to establish a clear result. This protocol followed up our previous observation (Hill et al, 2021 *eLife* 2021;10:e68211) that the OFF state of the myosin-containing thick filaments of the myosin filament characteristic of resting muscle was not fully recovered at the time of mechanical relaxation after a short contraction elicited by 100ms of repetitive stimulation, a short tetanus. Since it was well known that the response of skeletal muscle to a single stimulus is enhanced following such a tetanus, a phenomenon known as post-tetanic potentiation (PTP), we hypothesized that the structural basis of PTP might be the incomplete recovery of the OFF state of the myosin-containing thick filaments.

The optimal conditions for producing PTP had been well established by mechanical experiments, and correlated with the phosphorylation of one of the components of myosin, its regulatory light chain (Zhi G *et al*. 2005. *PNAS* 102:17519-24), and we had already tested the relevant protocols in our home laboratory. We were therefore able to apply them in the two remaining shifts at ESRF, using isolated intact EDL muscles of the mouse mounted vertically at sarcomere length 2.4μm at ID02 in a temperature-controlled trough in oxygenated Krebs solution at 27°C, with the Eiger 2-4M detector positioned either at 4 m from the muscle to record the equatorial reflections, the myosin-based layer lines, and the myosin-based meridionals, in particular the interference fine-structure of the M3 reflection, and the spacing of the M6 reflection that mediates mechano-sensing in the thick filament backbone, or at 31 m to record sarcomere length changes. The results of these experiments are currently being analysed.