ESRF	**Experiment title:** **Strain dependence of different force-generating transitions in actin-myosin molecular motor in muscle fibres**	**Experiment number**: SC2397
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Shifts:	**Local contact(s)**: Dr. Theyencheri NARAYANAN	*Received at ESRF:*

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Report:

Time on ID02 was devoted to:

1. Evaluation of our new experimental set-up for holding fibres vertically.
2. Evaluation of the effect of EDC cross-linking on the diffraction pattern of skeletal muscle fibres
3. Investigation of diffraction pattern from muscle fibres in which the Essential Light Chain (ELC) had been exchanged with a gold-labelled ELC.

1. Muscle fibres oriented vertically in the x-ray beam give diffraction patterns with better resolution along the meridian and row-lines. This has advantages as demonstrated by the team of Irving and Lombardi who demonstrated the resolution of interference patterns on the meridional reflections when using single intact fibres. We showed previously that interference fringes can be detected with horizontally mounted fibres, but the quality was not sufficient for useful measurements. We set about constructing an instrument that would allow mounting permeabilised fibres vertically. This required complete re-engineering of the apparatus. A requirement of the instrument is the ability to change the solution in which the fibre is immersed, whilst controlling fibre length and measuring fibre force and the sarcomere diffraction pattern. In addition, activation of contraction needs to be achieved rapidly (we choose the temperature-jump method), the x-ray path in the aqueous medium has to be minimized and the fibre temperature needs to be controlled.

   We successfully installed and tested the new instrument. The figure below compares the meridional diffraction patterns obtained from permeabilised muscle fibres of the rabbit psoas muscle, held in the x-ray beam at ID02, either in the vertical (thick line), or horizontal (thin line) orientation. The main features are labelled. All peaks are sharper in the vertical fibre. The minor layer line peaks such as M1 and M2 are resolved in the vertical, but not in the horizontal fibre.

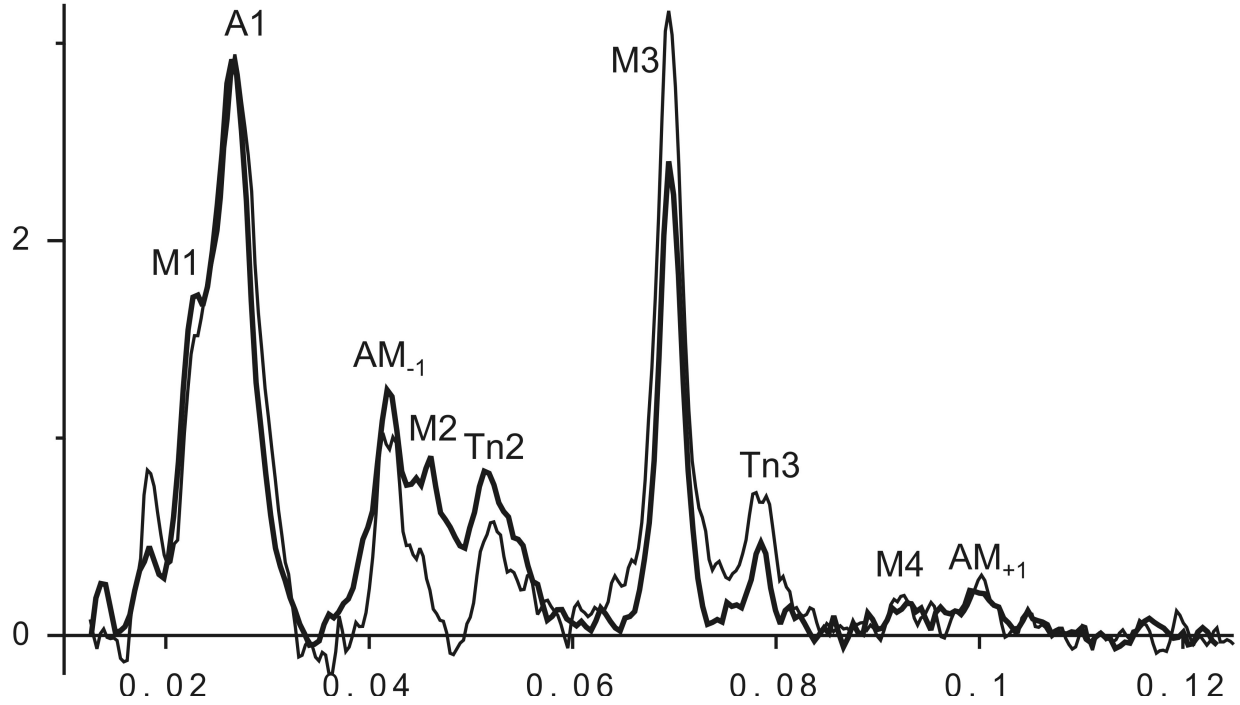

Figure 1: two rigor patterns collected from single fibres each: horizontal (thin line, 400 ms exposure, 2.5 m-long camera) and vertical (thick line, 200 ms exposure, 2.1 m-long camera). Although signal to noise ratio was better for the horizontal fibre mainly due to the longer exposure, the meridional resolution for the vertical fibre was better despite shorter camera.

The new set-up will allow us to investigate time-resolved changes in the interference patterns of the M3 and M6 reflections, something which has not been achieved previously with permeabilised fibres. The interference fringes provide information about the longitudinal orientation of the myosin cross-bridge with nanometre-scale resolution. These measurements will be important for a more complete description of the structural changes involved in force generation by muscle. In particular, we shall investigate the change in average cross-bridge orientation as the fibre temperature increases from 5 to 37°C when a T-jump is applied during isometric contraction.

2. Following tests of the new set-up, we set out to investigate the effect of EDC cross-linking on the diffraction pattern. We routinely use 'low-dose' EDC cross-linking treatment to stabilise the structure of muscle fibres during contraction. This procedure in which a small fraction of cross-bridges becomes covalently bound to their actin target sites results in the loss of calcium regulation of contraction and the loss of long-range shortening, but does not cause deterioration of the physiological response of muscle fibres during isometric contraction. Cross-linking provides several important advantages: the structural integrity of the fibres remains very high, even during prolonged contraction or contractions at physiological temperature. In our experience the diffraction pattern of isometrically contracting fibres is not altered by cross-linking. However this point is often questioned by our referees and it was felt that direct comparison of the diffraction patterns obtained with native and cross-linked fibres would be helpful. We obtained diffraction patterns at 5°C in the active state for native and cross-linked patterns. No measurable differences were observed. We were not able to compare the patterns at physiological temperature because the T-jump apparatus was not installed in this run, but this is one specific aim for our next beam time application.

3. The essential light chain of myosin (ELC) in permeabilised muscle fibres can be exchanged with a native ELC or with a genetically engineered light chain. This technique has been refined in the home laboratory and has enabled the introduction of fluorescent probes in contracting fibres. We adapted the procedure to introduce gold-labelled ELC into muscle fibres. The purpose of these experiments was to determine the effect of gold particles on the diffraction pattern. In principle this approach

should provide phase information about x-ray reflections influenced by the light chain. In practice, we were unable to detect changes in the diffraction pattern that could be attributed to gold-labelling, when compared to sham-exchanged fibres. Deterioration of the pattern was nevertheless observed as exchange conditions involve a high temperature incubation. We do not know at this stage whether the failure of the experiment was caused by problems with the gold-labelling or ELC exchange procedures, or whether the extent of gold incorporation is below that needed for resolution. Further control experiments will be carried out in the home laboratory before further attempts at the synchrotron.

The work described above deviates from the work initially proposed. This results from the fact that analysis of our data shows that data collected in SC1810 and SC2040 with an experimental protocol involving stretches and releases at 20 °C were of sufficient quality and did not require additional experiments. These data are now incorporated into a paper submitted for publication. The priority therefore changed towards the next stage of our work, the development of an experimental protocol involving vertically mounted fibres (see §1 above) and exploring gold labelling (see §2 above).

Recent relevant publications:

Bershitsky S.Y., Ferenczi M.A., Koubassova N.A., Tsaturyan A.K.(2008) Insight into the actin-myosin motor from X-ray diffraction on muscle *Frontiers in Bioscience* Accepted for publication.

Koubassova N.A., Bershitsky S.Y., Ferenczi M.A., Tsaturyan A.K.(2008) Direct Modeling of X-Ray Diffraction Pattern from Contracting Skeletal Muscle *Biophysical Journal* BIOPHYSJ/2007/120832 PMID: 18539638. In Press.