

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	Experiment title: STRUCTURE-FUNCTION RELATION OF THE MOLECULAR MOTOR IN MUSCLE: A TIME-RESOLVED X-RAY DIFFRACTION STUDY ON SINGLE MUSCLE FIBRES	Experiment number: SC-1388
Beamline: ID02	Date of experiments: III from: 16.3.2005 to: 21.3.2005	Date of report: 27.2.2006
Shifts: 15	Local contact(s): Pierre Panine	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> *Vincenzo Lombardi *Gabriella Piazzesi *Marco Linari *Massimo Reconditi *Ravikrishnan Elangovan *Elisabetta Brunello *Malcolm Irving </div> <div style="width: 55%;"> Laboratorio di Fisiologia, DBAG c/o Dipartimento di Fisica Via G. Sansone, 1 50019 Sesto Fiorentino (FI) Italy King's College London New Hunt's House Guy's Campus London SE1 1UL UK </div> </div>		

Report:

The aim of this project is to investigate the structural dynamics of the molecular motor of muscle, the myosin heads that cross-link the myosin and the actin filament, responsible for the generation of force/shortening in muscle and for the braking action of muscle during forcible lengthening. The investigation is made combining fast mechanics and X-ray diffraction in single fibres isolated from the frog skeletal muscle, where the molecular mechanism of contraction can be studied in the native system. Thanks to the collimation of the X-ray beam at ID2, ESRF, we can exploit the X-ray interference between the two arrays of myosin heads in the thick filament, to measure the motion of the myosin heads with sub-nanometre resolution. The experiments during SC-1388 were aimed at defining structural changes in the myosin heads and in the myosin and actin filaments (1) during the synchronous execution of the isotonic working stroke following step reduction in force superimposed on isometric contractions; (2) during the process of activation and force development in the isometric contraction; (3) during the force enhancement by stretch that is responsible for the efficient braking action of the active muscle in eccentric contractions. In the experiments of previous allocation periods (I and II), we completed experiments concerning the 1st and 3rd question of this LTP and started to address the 2nd question, the discrimination of structural changes in the myofilaments and myosin heads during activation and rise of isometric force. We separated the effects of activation from those of force generation by comparing meridional and layer line reflections collected during the isometric force development with those collected while preventing force generation with a ramp shortening at the unloaded shortening velocity (V_0). In this 3rd allocation period we focussed on changes of meridional and layer line reflections during rise of isometric force and force redevelopment after unloaded shortening of amplitude 50 or 100 nm per half-sarcomere.

Methods:

Single fibres from the tibialis anterior muscle of *Rana temporaria* were horizontally mounted in a trough containing Ringer solution at 4 °C and at ~2.2 μ m sarcomere length between a force transducer and a loudspeaker coil motor as already described (Linari et al., PNAS 97:7226, 2000). 5 ms time frames were collected using a CCD FReLoN detector (ESRF, Grenoble, France) and a 3 m camera length (so as to collect

up to the sixth order of the myosin based meridional reflections, M6) during the tetanus rise, during unloaded shortening ($V_0 = 2.62 \pm 0.05 \mu\text{m/s/hs}$) of 5% and 10% the fibre length (l_0) imposed on the isometric tetanic tension (T_0), and during the tension redevelopment following the end of unloaded shortening. Data analysis was performed using SAXS Package (P. Boesecke, ESRF), Fit2D (A. Hammersley, ESRF) and Igor Pro (WaveMetrix, Inc.). Mechanical data were collected and analysed with LabVIEW software.

Results:

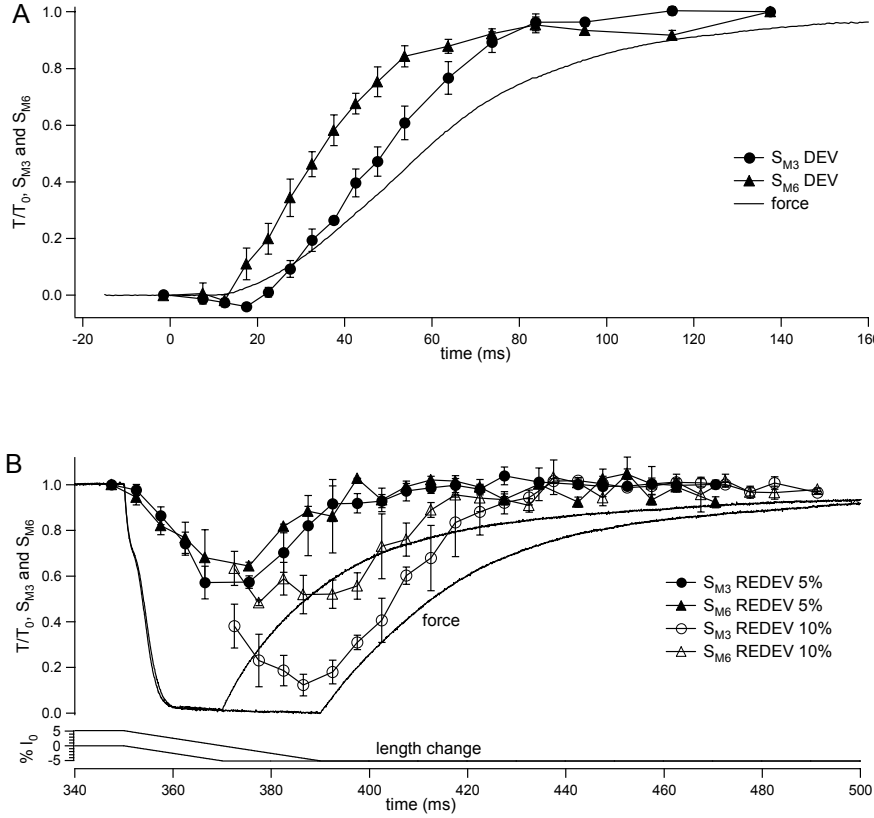


Fig. 1: Time course of spacing of M3 reflection (circles) and M6 reflection (triangles) during the development of the isometric tetanus (A, DEV) and during unloaded shortening followed by tension redevelopment (B, REDEV, filled symbols 5% l_0 shortening; open symbols 10% l_0 shortening). Changes in spacing are made relative to the isometric plateau value. Absolute S_{M3} values were 14.34 nm (rest) and 14.57 nm (T_0). Absolute S_{M6} values were 7.16 nm (rest) and 7.28 nm (T_0). Data in A from 5 fibres (mean \pm S.E.M.), force trace obtained averaging those from the 5 fibres. Data in B from 2 of the 5 fibres in A.

As shown in Fig.1, during the initial isometric force development the change of S_{M6} ($t_{1/2} = 23.1 \pm 4.3$ ms, mean \pm SD, 5 fibres) leads rise in force ($t_{1/2} = 48.3 \pm 6.4$ ms) much more than the change in S_{M3} ($t_{1/2} = 39.8 \pm 4.8$ ms). S_{M3} slowly decreases during unloaded shortening, reaching a value (14.47nm) mid-way between the plateau and resting values at the end of the 5% l_0 shortening, and continues to decrease toward the resting spacing till the end of the 10% l_0 shortening (14.37 nm); S_{M3} recovers with force at the end of the ramp. S_{M6} , during unloaded shortening, reduces less than S_{M3} , reaching a value (7.21 nm) mid-way between the plateau and resting values at the end of 10% l_0 shortening. The intensity of M3 decreases suddenly at the ramp start, while the intensity of M6 does not show significant changes within the limits of the present signal-to-noise ratio, confirming that this reflection is not sensitive to mechanical manoeuvres (Reconditi et al., Nature, **428**, 578-581, 2004). Plots of the relative spacing changes against relative force for the M3 and M6 reflections emphasises that S_{M6} changes lead force more than S_{M3} changes and the lead is the same independent of the two times higher rate of force rise during redevelopment with respect to the initial development of force. The changes in spacing of M3 and M6 reflections during the rise of isometric tetanus and during the force redevelopment from 10% l_0 unloaded shortening can be interpreted in terms of the intensity changes of two components with spacing characteristic of the resting and active spacings; only the active IM3 component is directly proportional to force demonstrating that it mainly originates from the force bearing heads.

Publications from these experiments:

- E. Brunello, G. Piazzesi, M. Linari, P. Bianco, M. Reconditi, P. Panine, W. Helsby, M. Irving and V. Lombardi. Time course of formation of myosin cross-bridges in tetanized single fibres from frog muscle measured by X-ray diffraction. *Biophys. J.* **86(1)**:564a 2925-Pos, 2004.
- E. Brunello, M. Reconditi, P. Bianco, M. Linari, P. Panine, T. Narayanan, W. Helsby, G. Piazzesi, M. Irving and V. Lombardi. A parallel elasticity observed by x-ray diffraction in tetanized single muscle fibres. *J. Muscle Res. Cell Motility* **25**, 246, 2004.
- M. Irving, M. Reconditi, M. Linari, L. Lucii, P. Bianco, V. Decostre, A. Stewart, Y.-B. Sun, P. Boesecke, T. Naraynan, T. Irving, G. Piazzesi, and V. Lombardi. X-ray interference studies of the myosin motor mechanism in single muscle fibres. *J. Muscle Res. Cell Motility* **25**, 243, 2004.
- E. Brunello, M. Reconditi, M. Linari, R. Elangovan, P. Panine, T. Narayanan, G. Piazzesi, V. Lombardi and M. Irving Spacing changes in the myosin based X-ray reflections during isometric force development. *Biophys. J.* **90**:427a, 2073-Pos, 2006.