

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: from: 21.4.2004 to: 26.4.2004	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 *Valerie Decostre *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 are 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. During previous LTP (SC-885), by using the interference X-ray diffraction method, we showed that the rapid force development that follows a shortening step in an active muscle fibre is due to a ~ 10 nm working stroke in the myosin head domain while it remains attached to actin, rather than to rapid ‘jumping’ of the heads between different monomers in the actin filament (Piazzesi et al., *Nature*, 415:659, 2002). However, in the force transient, the estimate of the axial motion of the myosin heads was complicated by the change in strain of the actin and myosin filaments. This complication is removed with the new protocol consisting in measuring structural changes associated to the velocity transient following a step reduction in load superimposed on the isometric contraction (Piazzesi et al., *J. Physiol.* 545:145, 2002).

Methods:

Single fibres from the tibialis anterior muscle of *Rana temporaria* were vertically mounted in a trough containing Ringer solution at 4 °C and at ~2.2 μm sarcomere length between a force transducer and a

loudspeaker coil motor as already described (Linari et al., PNAS 97:7226, 2000). 2D X-ray patterns were collected on the FReLoN CCD detector placed at 3 or 10 m from the specimen. Adequate time resolution to isolate the short lived transients following step perturbations in length or force was attained by generating short time windows (up to 100 μ s) with two fast shutters (LS 500, \sim 20 μ s switching time) in series in front of the preparation. For each step, trains of steps were imposed at the plateau of isometric tetanic contractions to increase the signal to noise ratio by adding corresponding time windows. For each timeframe, the exposure time was 5-10 ms per tetanus. To distribute the radiation damage, the fibre and the stage were shifted along the fibre axis by 200 μ m after each tetanus by using a remote controlled motor. Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF) and Peakfit software package (SPSS Inc.).

Results:

In the experiments completed in the first period of this LTP, we used step perturbations in force to synchronise the action of myosin heads and directly measure their axial motion at constant load and thus without the influence of filament compliance. We demonstrated that the working stroke is ca 11 nm at low load and is smaller and slower as the load is increased. These results are important to define the molecular determinant of the high efficiency in energy conversion by muscle and are published in a paper in Nature (Reconditi et al., Nature, 428:578, 2004). During the same experimental visit we also improved the statistics for the experiments devoted to study the structural changes accompanying the force enhancement by stretch, that has been attributed to fast cross-bridge recruitment on the basis of changes in mechanical stiffness. At the end of the elastic response following step stretches of 2-6 nm per half-sarcomere, the change in the fine structure of the M3 reflection is smaller than that expected from the imposed filament sliding, indicating a very fast attachment of the partner head of the same myosin molecule on the next actin monomer 5.5 nm apart in the M-ward direction. The results have been presented at the Biophysical Society conference.

Publications on these experiments:

M. Reconditi, M. Linari, L. Lucii, A. Stewart, Y.-B. Sun, P. Boesecke, T. Narayanan, R.F. Fischetti, T. Irving, G. Piazzesi, M. Irving and V. Lombardi. The myosin motor in muscle generates a smaller and slower working stroke at higher loads. *Nature*, **428**, 578-581, 2004.

Lombardi, V., Piazzesi, G., Reconditi, M., Linari, M., Lucii, L., Stewart, A., Sun, Y.-B., Boesecke, P., Narayanan, T., Irving, T. and Irving M.- X-ray diffraction studies of the contractile mechanism in single muscle fibres. *Phyl. Trans. R. Soc. B* **359**, 1883-1893, 2004.

Reconditi, M., Linari, M., Lucii, L., Stewart, A., Sun, Y.-B., Narayanan, T., Boesecke, P., Irving, T., Piazzesi, G., Irving, M. and Lombardi, V. - Structure-Function Relation of the Myosin Motor in Striated Muscle. *Ann. N.Y. Acad. Sci.* **1047**, 232-247, 2005.

M. Reconditi, L. Lucii, E. Brunello, M. Linari, Y.-B. Sun, T. Narayanan, P. Panine, G. Piazzesi, M. Irving and V. Lombardi. X-ray interference study of stretch-induced cross-bridge attachment in active single fibres from frog muscle. *J. Muscle Res. Cell Motil.* **25**, 246, 2004.

M. Irving, M. Reconditi, M. Linari, L. Lucii, P. Bianco, V. Decostre, A. Stewart, Y.-B. Sun, P. Boesecke, T. Narayanan, T. Irving, G. Piazzesi, and V. Lombardi. X-ray interference studies of the myosin motor mechanism in single muscle fibres. *J. Muscle Res. Cell Motil.* **25**, 243, 2004

M. Reconditi, E. Brunello, R. Elangovan, P. Panine, T. Narayanan, M. Irving, M. Linari, G. Piazzesi and V. Lombardi. Myosin cross-bridge recruitment by stretch occurs on the submillisecond timescale. *Biophys. J.* **90**, 106a, 493-Pos., 2006