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ESRF

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.

ESRF	**Experiment title:** *THE MOLECULAR MOTOR IN MUSCLE STUDIED WITH TIME-RESOLVED X-RAY DIFFRACTION ON SINGLE MUSCLE FIBRES*	**Experiment number**: SC-2051
Beamline:	**Date of experiment**: from: 06.12.2006 to: 12.12.2006	**Date of report**: 15.01.2008
Shifts:	**Local contact(s)**: Pierre Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Vincenzo Lombardi, *Gabriella Piazzesi, *Marco Linari, *Luca Fusi, *Elisabetta Brunello, *Marco Caremani, *Massimo Reconditi, Mario Dolfi, Laboratorio di Fisiologia, DBAG, c/o Dipartimento di Fisica, Università degli Studi di Firenze

*Malcolm Irving, The Randall Institute, King's College London

Report:

1) **Comparison of myosin motor conformations in isometric contraction and rigor**. We compared X-ray interference patterns at rest (all the motors lying on the myosin filament), during isometric contraction (30 % of motors are attached to actin in a conformation close to the beginning of the working stroke) and in rigor (all motors attached to actin in a conformation at the end of the working stroke) in single fibres isolated from the skeletal muscle of the frog (Rana temporaria). Our aims were to describe the structural changes in the array of motors working in parallel in the muscle sarcomere and in the supporting actin and myosin filaments during activation and force generation, and to compare the motor attachment pattern in isometric contraction, when one motor domain of each myosin molecule is attached to actin, with that in rigor, when both motor domains are attached. The rigor state was induced using a new protocol that does not require skinning of the fibre or attachment to the transducer levers via damaged ends. Intact fibres were mounted in a multidrop system for rapid solution exchange (Linari et al., Biophys. J. 74:2459, 1998) and then transferred into an experimental trough that could be sealed for vertical fibre mounting. Consistent X-ray data in rigor were collected at low static tension for comparison with that from the resting and contracting states, and analysis of these data is in progress. Further experiments are required to collect X-ray patterns at high static tension and the dynamic responses to length steps in rigor, to determine the elastic and viscoelastic properties of the motors and myofilaments. Based on the experience gained during the beamtime we are now developing a new apparatus for transferring fibres between troughs on the beamline while preserving the mechanical integrity of the fiber-tendon connections.

2) **X-ray interference studies of mammalian skeletal muscle fibres**. We demonstrated that useful X-ray interference data (Fig. 1) could be recorded at ID02 from horizontally mounted skinned fibres of mammalian skeletal muscle mounted in an apparatus that allows combined X-ray and sarcomere-level mechanical measurements (Linari et al., J. Physiol. 473:8P, 1993; J. Physiol. 554:335, 2004; Biophys. J. 92:2476, 2007). This result opens up a new programme of research that has the potential to relate the mechanical and structural steps in the myosin motor to the chemical transitions in the actomyosin ATPase cycle. The spatial

resolution, signal:noise and sensitivity of the X-ray camera/detector system were significantly improved compared to previous experiments at ID02 by the combination of (1) the new FReLoN CCD detector (active area 10 x10 cm^2, pixel size 52 µm, possibility of binning up to 8 pixels in the direction perpendicular to the fibre axis); (2) reduction of the horizontal width of the beam to 0.3 mm (FWHM) at the expense of decreasing incident intensity from 3.10^{13} to 10^{13} photons/s. In this way, the fine structure of the M3 reflection could be resolved in horizontally mounted fibres with a camera length of 7m. As a preliminary experiment in the new programme we investigated the temperature sensitivity of the structural order of mammalian fibres in the relaxed state in the range 4-20 °C.

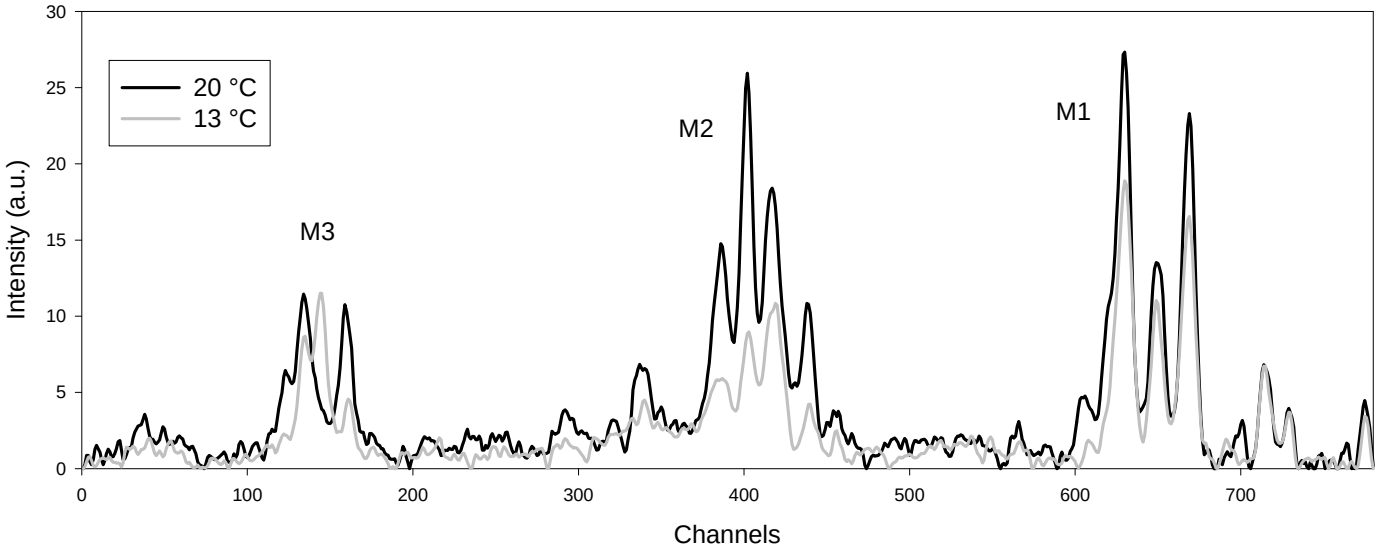

Fig. 1. Axial intensity distributions of the meridional reflections in the relaxed state at 13 °C (grey line) and 20 °C (black line) from a bundle of three skinned fibres from rabbit psoas muscle mounted horizontally. Each trace is from 100 ms X-ray exposure.

Publications from ESRF experiments during the last 2 years:

E. Brunello, P. Bianco, G. Piazzesi, M. Linari, M. Reconditi, P. Panine, T. Narayanan, W. Helsby, M. Irving and V. Lombardi - Structural changes in the myosin filament and cross-bridges during active force development in single intact frog muscle fibres: stiffness and X-ray diffraction measurements. *J. Physiol.*, **577.3**, 971-984, 2006.

M. Reconditi. Recent improvements in small angle x-ray diffraction for the study of muscle physiology. *Rep. Progr. Phys.* **69**, 2709-2759, 2006.

E. Brunello, M. Reconditi, R. Elangovan, M. Linari, Y.-B. Sun, T. Narayanan, P. Panine, G. Piazzesi, M. Irving, V. Lombardi - Skeletal muscle resists stretch by rapid binding of the second motor domain of myosin to actin. *Proc. Natl. Acad. Sci. U S A.* **104**(50):20114-20119, 2007

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.

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

M. Reconditi, M. Linari, E. Brunello, L. Fusi, T. Narayanan, P. Panine, G. Piazzesi, M. Irving, V. Lombardi. (2007). Interfilamentary spacing in intact fibres from frog muscle at rest, durino active contraction and in rigor. *2007 Biophys. Soc. Meeting Abstract. Biophys. J., Suppl.,* 374a, Abstract, 1766-Pos.