Fig. 2: Relative 3M/6M intensity ratio vs. sarcomer length: theory and experiment

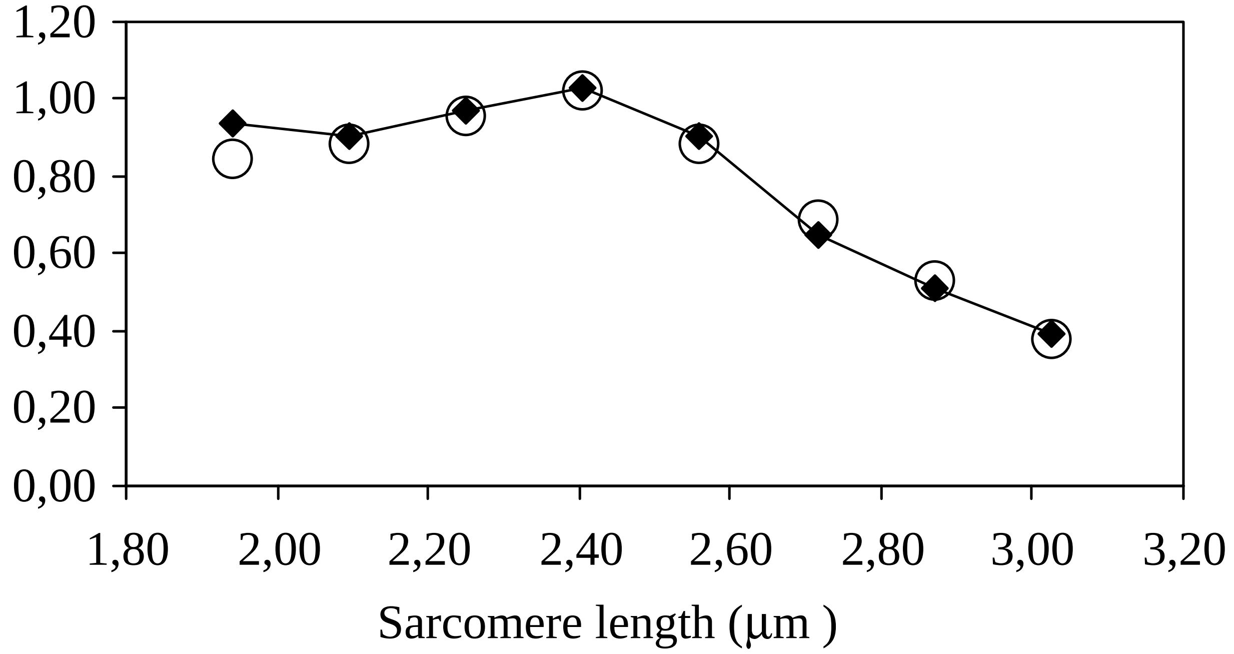
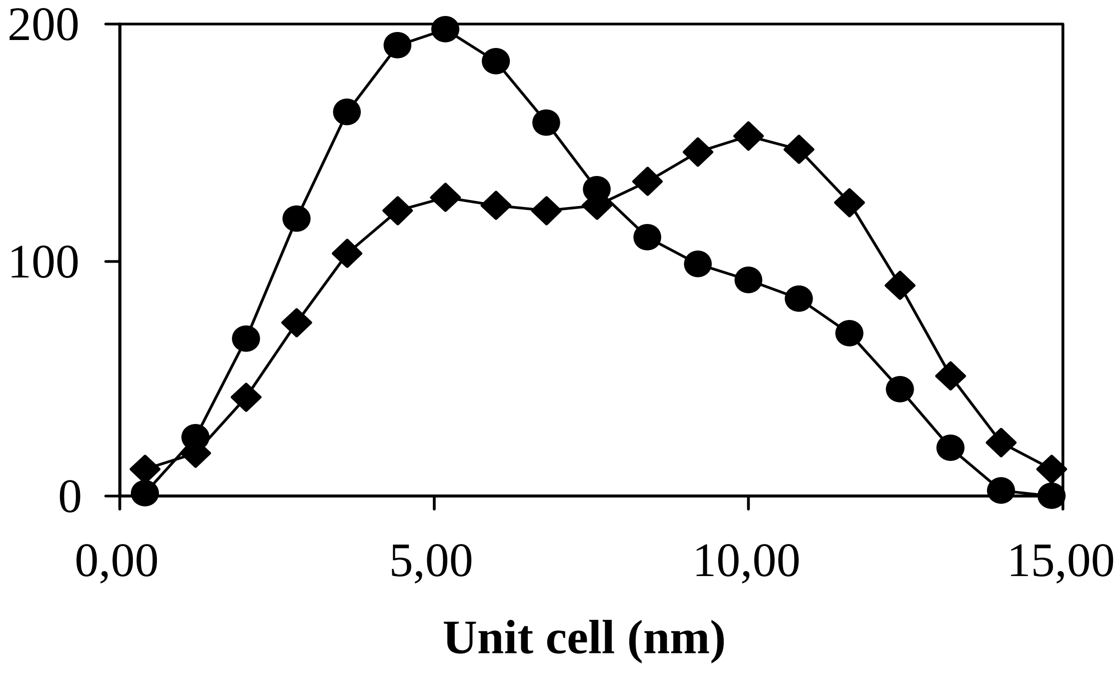


Fig. 3: Axial mass projection of myosin heads at rest (diamonds) and at Po (circles)



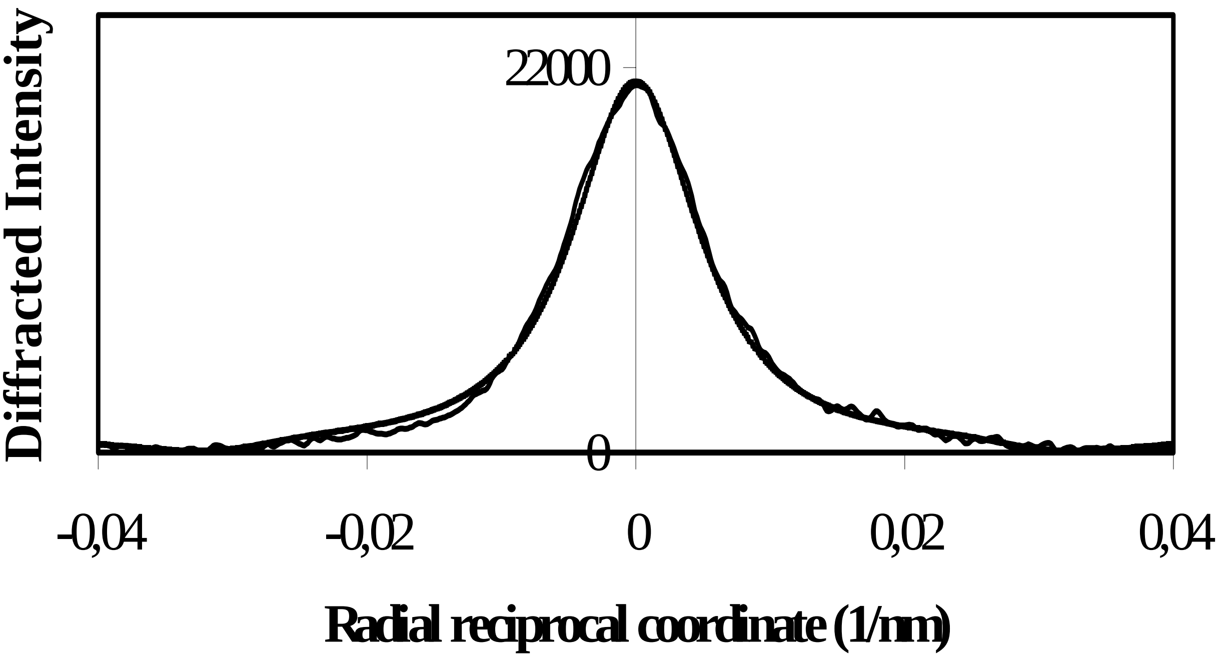


Fig. 1:Radial profile of the 3M: theory and experiment

d) Finally, we have begun to address the new lines of work presented in the original proposal. We recall that these were to study the structural events associated with thin filament activation. These are difficult experiments because the activation occurs in the first few milliseconds after the initiation of the contractile cycle and, moreover, the intermediate states that one wishes to capture are also short-lived. In addition, the reflections associated with these phenomena are not particularly strong. We have collected some preliminary results on the troponin activation phenomenon. These results suggest that it is possible to define/detail the axial changes in the troponin reflections that are associated with activation. This is done by exploiting once again the presence of pronounced interference effects. We also proposed to work on tropomyosin. This has not yet started but we hope to get onto it soon.

Relevant references:

1. Design, implementation and methods for the manufacture of components of a two-dimensional X-ray detector. I. Ramos-Lerate, D. Beltrán, I. Magrans, J.C. Martínez, J.A. Perlas, J. Bordas. Nuclear Instruments and methods in Physics Research, section A. 2002 (in press).

2. Suitability study of a histogramming software architecture in the data acquisition system of position sensitive X-ray detectors. I. Magrans*, J.A. Perlas, D. Beltrán, I. Ramos-Lerate, J.C. Martínez, J. Bordas. Nuclear Instruments and methods in Physics Research, section A. 2002 (in press).

3. Axial Disposition of Myosin heads in Isometrically Contracting Muscles. J. Juanhuix, J. Bordas, J. Campmany, A. Svensson, M.L. Bassford and T. Narayanan. Biophysical Journal Vol 80 1429-1441 (2001).

4. Structure and Mechanics of Muscle and Tendon. P. Fratzl and J. Bordas. Synchrotron Radiation News Vol. 15, No. 4 18-26(2002).

intensity profiles need to be fitted with a theory that includes both crystallographic and helical disorder (Fig. 1). Then the measured variations of the integrated intensities of the experimental data can be accounted for in terms of disorder. The two lines in Fig. 1, that are practically superimposed, illustrate how the shape, therefore the intensity, of the 3M layer line can be reproduced by the appropriate theory. This is essential to obtain the appropriate intensity of the underlying helically diffracting repeating motif (i.e. the thick filament in this case) which combined with the phases can yield interpretable electron density maps. Fig. 2 illustrates how the different intensity dependence on sarcomere length of the important third and sixth myosin layer lines (3M and 6M, respectively) during isometric contraction can satisfactorily be accounted for. The figure shows how this hitherto un-explained dependence on sarcomere length (diamonds) can be accounted for by inclusion of disorder effects in the muscle lattice (open circles). Only after appropriate corrections of this nature are applied it is possible to compare electron density maps describing the myosin heads in different intermediate structural states (see example in Fig. 3). Considering the complexity of the theory it is gratifying that the results can be so accurately accounted for (manuscript in preparation).

c) Similar calculations have been applied to the data collected at rest, during shortening and stretches. The combination of phase extraction and intensity determination has yielded a number of electron density maps that can be quantitatively compared. This is illustrated in Fig. 3 where electron density maps, corresponding to the mass projection at Po (isometric plateau) and at rest, are shown. The two lobes of density represent the mass projection of a pair of myosin heads in the unit cell, therefore one can conclude that whilst at Po (fully developed tension during isometric contraction) one head in the pair adopts a more perpendicular orientation to the muscle axis (ref.3), at rest the configuration of the two heads is more alike. We have found that the maps during isotonic shortening are essentially identical to those at rest as long as the load is negligible, whilst during stretches they are much like those at Po. Note that the total intensity in the mass projections in Fig.3 are identical within error. This testifies for the validity of the procedure for intensity evaluation and correction. The biological significance of these results will be discussed elsewhere (manuscript in preparation).

conditioning of the specimens. In order to improve the experimental duty cycle, the equipment was modified so that two teams (ourselves and the Hannover team, Dr. T. Kraft and colleagues) could install their physiological apparatus in the hutch. These can be quickly, and reproducibly, moved into the beam whenever one team is ready to carry out their experiments. The result is that, after some initial teething troubles, the duty cycle of the experiments was almost doubled. Overall this works very well.

Scientific output:

a) We have developed experimental and theoretical methods to obtain phase information for the meridional reflections (i.e. those that contain the information about the axial configuration of the myosin heads) from the interference phenomena present in the X-ray patterns (refs. 3,4). These measurements have now been extended to the recording of diffraction diagrams from muscles undergoing various forms of contraction, e.g. unloaded shortening, shortening under various loads, as well as under controlled stretches. The relevant phase information has been obtained, albeit at a relatively low resolution of ca. 5.0 nm. This is because the size of the detectors available is not large enough to cover the reciprocal space range needed to extend the data to high resolution and at the same time resolve the interference effects. Until more suitable detectors come along, this part of the work must be regarded as complete.

b) On the other hand, we have found that in order to compare the conformation of the myosin heads in the various forms of contraction it is also necessary to include fairly sophisticated intensity corrections. These are essential to obtain electron density maps that can be quantitatively compared with each other. The reason for this is that the muscle diffraction patterns are susceptible to crystallographic disorder effects, which are strongly influenced by the exact contracting condition of the muscle (i.e. resting, isometric or isotonic). To solve this problem we have developed what seems to be a sound/rigorous theoretical approach. The application of these theoretical corrections requires time resolved data in which the peak shapes are very accurately determined. This accuracy can only be achieved with the beam profile only available from a third generation X-ray source such as the ESRF. The combined experimental/theoretical work has allowed us to extract the necessary corrections for a number of contracting states. The attached figures illustrate the procedure. The

Report:

The conditions of a Long Term Project (LTP) are that: in addition to tackling a scientific program of value, the participants must engage in the development of methods and/or instrumentation that could be, in due course, of benefit to the ESRF user community, and; to improve the efficiency in the use of the ESRF. Therefore, this report is in two parts: the first refers to what we have been doing about the technical aspects of our use of the ESRF, and; the second is concerned with progress in muscle research.

Technical/instrumentation aspects:

a) In order to explore the possibility to install easily accessible detectors of different kinds to those normally available (such as the RAPID detector on loan from the SRS, Daresbury), a by-pass of the large evacuated chamber in ID2 was installed by ESRF staff and tried out. This was done by installing horizontally diffracting monochromator crystals, so that the X-ray beam could be diverted to one side and by-pass the large evacuated chamber. The measurements clearly indicated that an unacceptable loss of useful intensity was suffered. This was due to the fact that the rocking curve width of the crystals in use was too narrow relative to the horizontal beam divergence. The solution to this problem is to replace the current crystals with asymmetrically cut ones as this would broaden their input angular acceptance. Hopefully this will be tried out sometime in the future.

b) In terms of developing equipment of general interest, one should mention that we are in the final stages of signing a protocol of collaboration with the ESRF. The aim is to construct and, in due course exploit, single photon counting position sensitive devices with the specific aim of enabling the pursuit of time resolved experiments. This should result in the technical possibility to carry out true kinetic experiments with time resolutions in the sub-millisecond domain. This is something that currently is not possible at the ESRF. In parallel other work in progress (refs. 1,2), we have additionally committed ca. 3 work-years/year and in excess of ca. 120,000 euros to this collaboration.

c) The efficiency of data collection has been improved significantly. In our line of work the duty cycle is limited by the physiological preparation. Typically a measuring protocol involves no more than a few minutes, whilst most of the time goes into the preparation and

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Beamline:	**Date of experiment**: 2 years long term project	**Date of report**: 10 October 2002
Shifts:	**Local contact(s)**: T. Narayanan	*Received at ESRF:*

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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.