EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** Structural basis of length dependent activation in the heart	**Experiment number**: LS-2512
Beamline:	**Date of experiment**: from: 11 Feb 2016 to: 16 Feb 2016	**Date of report**:
Shifts:	**Local contact(s)**: Theyencheri Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Introduction. The aim of this project is to investigate the structural basis of the Frank-Starling law of the heart, which states that the force during the contraction (systole) is adapted to the volume attained by the ventricle at the end of the relaxation (end-diastolic volume). At the level of the sarcomere, the structural unit of heart muscle cell, in which myosin motors work cooperatively generating steady force and shortening by cyclic ATP-driven interactions with the interdigitating actin filaments, the Frank-Starling law consists in the so called length-dependent activation (LDA), that is the increase in the force of contraction with the increase in sarcomere length (SL). Thus to collect and control the SL changes accompanying the mechanical output is a crucial prerequisite of the investigation. In the previous visit (LS-2450) we have combined the fast sarcomere mechanics developed in our lab for intact trabeculae with the unique possibility at beamline ID02 to vary the sample-to-detector distance from 0.6 to 30 m, so that the nanometer-scale signals originating from the two arrays of myosin motors in each thick filament can be recorded together with the micrometer-scale changes in the length of the sarcomeres interrogated by the X-ray beam. In this way we have found that the number of motors available for force generation is adjusted to the systolic force and SL, independent of the diastolic SL. The results are presented in a Manuscript submitted to Science. To understand the molecular basis of the LDA, here we have explored the structural changes induced on the thick myosin-containing filament by changes in SL in diastole, in the range of SL 1.9-2-3 μm..

Methods. The heart trabecula dissected from the rat ventricle was mounted in a thermoregulated trough perfused with oxygenated solution (1.2 ml/min, 27°C) and attached, via titanium double hooks, to the lever arms of a strain gauge force transducer and a loudspeaker motor carried on a moveable stage of a microscope. Sarcomere length (SL) was measured with a 40x dry objective and a 25x eyepiece. The length of the trabecula was adjusted to have an initial SL of ~2.1 μm (L0). The stage was then vertically mounted in the hutch. A FReLoN CCD detector was placed at 30 m from the preparation to collect the first orders of the sarcomeric reflections with 1.6 ms time windows. Shorter and longer SL were obtained by changing the trabecula length

by about ± 8% L0 and the corresponding sarcomeric reflections were recorded. The detector was then moved to 1.6 m to collect up to the 6th order of the myosin-based meridional reflections (time windows 10 ms) during diastole at the same trabecula lengths.

Results. The SL measured by the 2D patterns collected with 30 m camera length for the three different trabecula lengths were1.97 ± 0.01 µm, 2.11 ± 0.01 µm and 2.26 ± 0.01 µm, mean ± SE from 4 trabeculae. The patterns collected with 1.6 m camera length were normalised by the intensity of the equatorial 1,0 reflection at each SL to correct for the effects of the different diffracting mass in the X-ray path (Reconditi et al. 2014, J. Physiol. 592, 1119). The intensity distribution along the meridional axis of the X-ray pattern (parallel to the filament axis, Fig. 1) is dominated by the myosin-based M3 reflection, originating from the axial repeat of the myosin motors; the other myosin-based reflections are the M6 (from the backbone periodicity) and the M2, M4, M5 forbidden reflections, due to a regular axial perturbation induced by the Myosin Binding Protein C (MyBP-C, that also contributes to the M1 reflection). The spacings and the fine structure of all the myosin reflections did not change significantly in the range of SL explored, while their intensities (corrected by the radial width change) increased with the increase of SL. These results indicate that in diastole the thick filament is in the OFF state (most of myosin motors folded back towards the centre of the sarcomere and the thick filament with a short periodicity). The increase in SL induces both a further increase in the number of motors in the OFF state and a higher order of the MyBP-C.

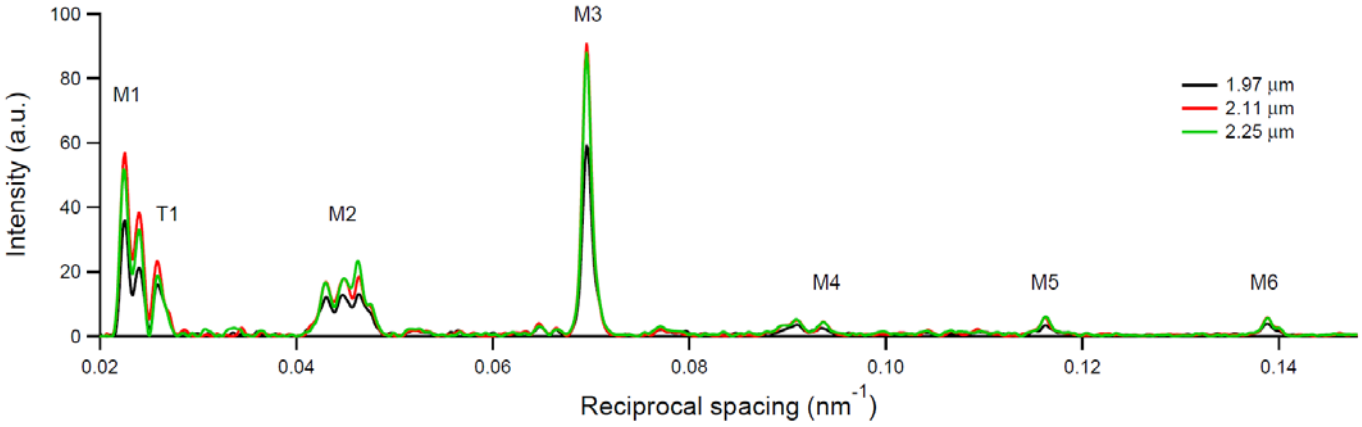

Fig. 1. Meridional intensity profiles from 4 trabeculae at three different SL, integration limits 0.011 nm^{-1} on either side of the meridional axis, intensity normalised for equatorial 1,0 intensity.