EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 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-2450
Beamline:	**Date of experiment**: from: 30 Sept 2015 to: 06 Oct 2015	**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. With this visit we started an investigation on the structural basis of the Frank-Starling law of the heart, the mechanism that allows the force during the contraction (systole) to be 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). A crucial prerequisite of this research is therefore to record and control the SL changes underlying the modulation of the mechanical performance and the related structural dynamics of motor and cytoskeletal proteins. For this fast sarcomere mechanics in intact trabeculae is combined with X-ray diffraction from synchrotron light, to achieve the mechanical and structural resolution adequate to define the function of the motor protein, while preserving the control of the sarcomere. This is possible due to the recent upgrade of the high brilliance beamline ID02 of the European Synchrotron (ESRF, Grenoble, France), which makes this beamline unique worldwide for the possibility 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 and from the double hexagonal lattice formed by the myosin and actin filaments can be recorded together with the micrometer-scale changes in the length of the sarcomeres interrogated by the X-ray beam.

Methods. The trabecula was vertically mounted in a thermoregulated trough (27°C), perfused with oxygenated physiological solution, and electrically paced at a frequency of 0.5Hz. A FReLoN CCD detector was placed at either 1.6 m from the preparation to collect up to the 6th order of the myosin-based meridional reflections, or 30 m to measure sarcomere length (SL) during diastole-systole cycles (see Fig 1). SL was held constant during force development (sarcomere isometric conditions) by feeding the motor-servo system with a signal based on the changes in SL recorded in the preceding

fixed-end contraction (Caremani et al., *PNAS*, **113**:3675-3680, 2016).

Results. In diastole the spacings of the M3 meridional reflection (SM3, associated with the myosin heads axial periodicity) and of its second order M6 (SM6, associated with the myosin-containing thick filament backbone periodicity) are 14.361 ± 0.004 (mean ± SD) and 7.195 nm respectively (SL 2.25 µm and $[Ca^{2+}]_o$ 2.5 mM, four trabeculae). At the maximum of systolic force attained with $[Ca^{2+}]_o$ 2.5 mM and SL 2.1 µm both SM3 and SM6 were ~1% larger. The M3 reflection was sampled by X-ray interference between half-sarcomeres, showing a dominant peak with two small satellites on either side in diastole, while at the maximum of systolic force in sarcomere isometric conditions it was split in two peaks of comparable size. These results indicate that in diastole myosin heads are folded back towards the thick filament midpoint in agreement with the OFF state of the filament in skeletal muscle (Zoghbi et al., *PNAS*, **105,** 2386–2390, 2008; Linari et al., *Nature*, **228**, 576-579, 2015). Consistent with the transition to the ON state described in the skeletal muscle thick filament, the development of systolic force is associated with the 1% increase of thick filament extension and the movement of the myosin heads by ca 10 nm away from the filament midpoint (Reconditi et al., *PNAS*, **108**, 7236-7240, 2011).

A paper is in preparation to report the first in situ description of the cardiac myosin dynamics during systole-diastole cycle.

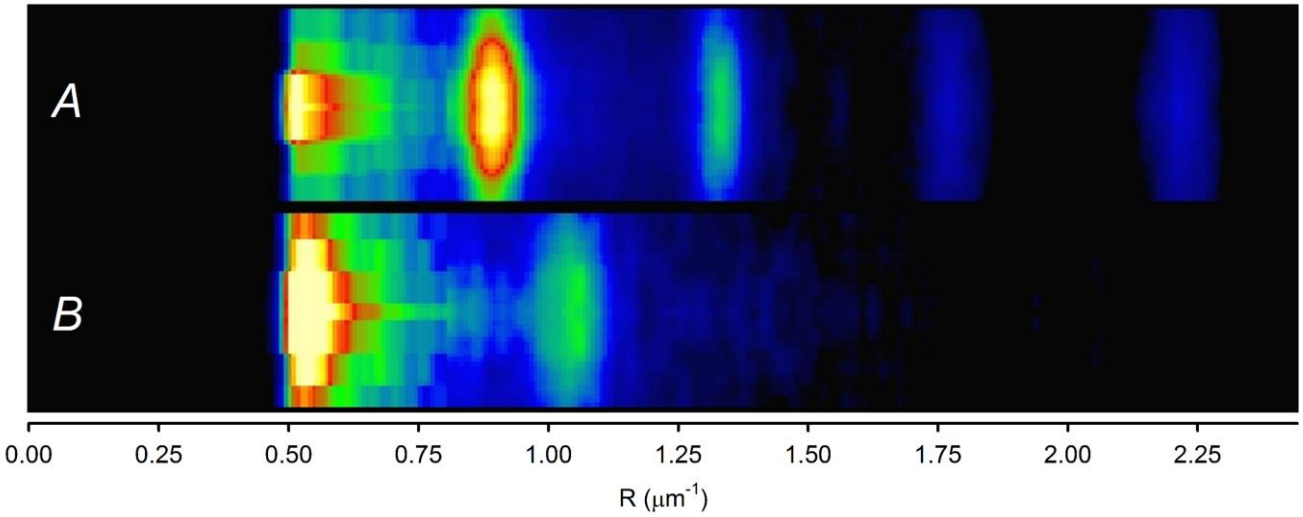

Fig. 1. X-ray diffraction patterns from a trabecula collected on a FReLoN CCD detector at 30 m (10 ms total exposure) to collect the first orders of sarcomere periodicity in diastole (A, SL 2.25 µm) and at the peak of force developed during the systole (B, SL 1.95 µm). The abscissa scale denotes reciprocal spacing.