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-2650
Beamline:	**Date of experiment**: from: 22 Feb 2017 to: 27 Feb 2017	**Date of report**:
Shifts:	**Local contact(s)**: Theyencheri Narayanan	*Received at ESRF:*

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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, stating 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 2 μm long structural unit of heart muscle cell, in which myosin motors, extending from the thick filament, work cooperatively to generate steady force and shortening by cyclic ATP-driven interactions with the interdigitating actin filaments, the Frank-Starling law consists in the increase of the force of contraction with the increase in sarcomere length, SL (length-dependent activation, LDA). Thus to record the SL changes accompanying the mechanical output together with the structural signals from the thick and thin filaments during the systole-diastole cycle is a crucial prerequisite of the investigation. This is achieved by the combination of fast sarcomere mechanics for intact trabeculae with the possibility offered at beamline ID02 to vary the sample-to-detector distance from 0.6 to 31 m to record both the nanometer-scale signals originating from the two arrays of myosin motors in each thick filament and the micrometer-scale changes in the length of the sarcomeres interrogated by the X-ray beam. In previous visits (LS-2576, Nov 2016; LS-2512, Feb 2016; LS 2450, Oct 2015) we showed that in the heart as in the skeletal muscle (1) a dual filament mechanism of regulation of contraction operates: the canonical Ca^{2+}-dependent thin filament activation, making the actin sites available for binding of the myosin motors, and the mechano-sensing in the thick filament (2), acting as a downstream gearbox that adapts to the load the recruitment of the myosin motors from their energetically convenient OFF state (3,4). In a heartbeat, unlike during skeletal muscle tetanic contraction, the rise of internal [Ca^{2+}] is transient and may not reach the level for full thin filament activation, thus the mechanical response depends on both the internal [Ca^{2+}] and the sensitivity of the thin filament to calcium (5,6), parameters that are under the control of several regulatory systems like LDA and phosphorylation of contractile, regulatory, and cytoskeletal proteins. During visit LS-2650 we aimed at investigating how the regulatory state of the thick filament in an electrically paced intact cardiac trabecula changes in diastole in relation to inotropic interventions like the increase in sarcomere length (SL) from 1.9 to 2.3 μm, obtained by either slowly lengthening the trabecula to avoid large passive force development or by imposing a force step up to 0.15 the force at the peak of an isometric twitch.

Methods. Heart trabeculae are dissected from the right ventricle of the rat and mounted in a thermoregulated trough perfused with oxygenated solution (1.2 ml/min, 27°C) between the lever arms of a strain gauge force transducer and a loudspeaker motor carried on the moveable stage of a microscope. SL is measured with a 40x dry objective and a 25x eyepiece. The length of the trabecula is adjusted to have an initial SL of ~2.1 μm (L_0 length). A pair of mylar windows is positioned close to the trabecula, about 1 mm apart, to minimize the X-ray path in the solution. The trough is sealed to prevent solution leakage and the trabecula is vertically mounted in the beam path. Trabeculae are electrically stimulated at 0.5 Hz to produce twitches. A FReLoN CCD detector is placed at 31 m from the preparation to collect the first orders of the sarcomeric reflections with 1.6 ms time windows. Change in SL (range 1.95-2.25 μm) is achieved by changing the trabecula length by about ± 8% L_0 and recording the corresponding sarcomeric reflections. Sarcomeric reflections are also recorded during the lengthening response to force steps imposed starting from SL of 1.95 μm, the amplitude of which is chosen to attain a final SL of ca. 2.25 μm. The detector is then moved to 1.6 m and the same manoeuvres are repeated to collect up to the 6th order of the myosin-based meridional reflections (5-10 ms time windows).

Results. The X-ray data collected with LS-2650, integrated with those of LS-2576, provided an adequate statistics for the demonstration that the spacing and fine structure of all the meridional myosin based reflections (the M1, also contributed by the Myosin Binding Protein C (MyBP-C), the M3 originating from the axial repeat of the myosin motors; the M6 from the backbone periodicity, and the M2, M4, M5 forbidden reflections due to an axial perturbation induced by the MyBP-C) do not change with increse in SL from 1.95 to 2.25 μm (accompanied by increse in passive force from 0 to 0.06 the force at the twitch peak (Tp) at 2.25 μm). The intensities of all reflections, corrected for the effects of the change of diffracting mass in the X-ray beam, increase by 10-15% with the increase of SL. A similar intensity increase is shown by the first order myosin layer line (ML1), originating from the three stranded quasi-helical symmetry of myosin motors on the surface of the thick filament. When the longer SL of 2.25 μm is obtained with a force step (complete in 10-40 ms) to 0.15 Tp, we did not find any changes in the spacing and fine structure of the meridional reflections with respect to both the shorter SL and the same SL with lower passive force (no step). The intensity of the reflections was about the same at the same SL independent of the passive force (0.06 Tp, no step, 0.15 Tp step). These results contradict recent X-ray results showing that SL affects the thick filament structure (7,8) and give further evidence to the view that the sarcomere length sensitivity modulating the performance of the heart in systole is not based on changes in the thick filament structure in diastole, reinforcing the new view of the Frank-Starling mechanism that a rapid stress-dependent thick filament regulation adjusts the energetic cost of the heart beat to the ventricular end-systolic pressure-volume relation, independent of the end-diastolic SL (2).

References. 1. Linari *et al*. 2015, *Nature* **528**:276-9; **2.** Reconditi *et al*. 2017, *PNAS* **114**:3240-5; **3.** Woodhead *et al*. 2005, *Nature* **436**:1195-9; **4.** Stewart *et al*. PNAS **107**:430-5; **5.** Allen and Kentish 1985, *J Mol Cell Cardiol* **17**:821-40; **6.** ter Keurs 2012, *Am J Physiol Heart Circ Physiol* **302**:H38-50; **7.** Farman *et al*. 2011, *Am J Physiol Heart Circ Physiol* **300**:H2155-60; **8.** Ait-Mou *et al*. 2016, *PNAS* **113**:2306-11.