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-2576
Beamline:	**Date of experiment**: from: 16 Nov 2016 to: 21 Nov 2016	**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, 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 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 in 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. During visit LS 2576 (Nov 2016), as in the previous visits LS 2450 (Oct 2015) and LS-2512 (Feb 2016) we have combined the fast sarcomere mechanics for intact trabeculae developed in our lab in Florence with the possibility offered at beamline ID02 to vary the sample-to-detector distance from 0.6 to 30 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. The results of the experiments brought to important conclusions in relation to the effect, on the X-ray signals marking the state of the thick filament, of the diastolic SL (range 1.9-2-3 μm), and in relation to the dependence of thick filament activation on the systolic force and SL.

Methods. The heart trabecula, dissected from the right ventricle of the rat, is 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 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 (L0). 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 either in fixed end (FE) conditions or in sarcomere length clamp (LC) conditions (Caremani et al. 2016). A FReLoN CCD detector is placed at 30 m from the preparation to collect the first orders of the sarcomeric reflections with 1.6 ms time windows. In diastole, shorter (~1.95 μm) and longer (~2.25 μm) SL are obtained by changing the trabecula length by about ± 8% L0 and the corresponding sarcomeric reflections are recorded. X-rays patterns are recorded also at the peak of both FE and LC twitches (Fig. 1A,C). The detector is then moved to 1.6 m to collect up to the 6th order of the myosin-based meridional reflections (5-

10 ms time windows) at the same trabecula lengths (Fig. 1B,D) as those set for the 30 m frames.

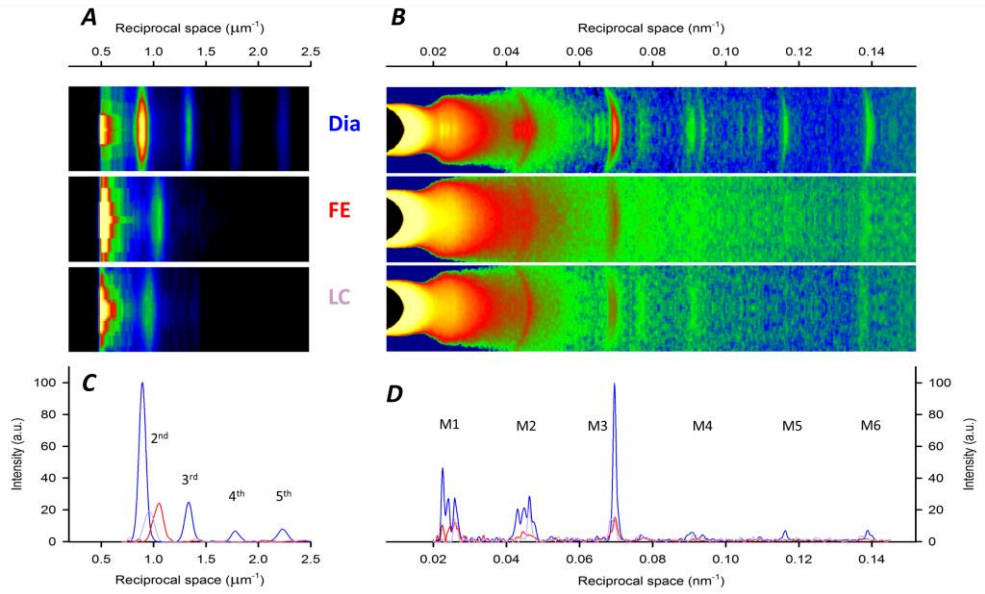

Fig.1 ***A.*** Meridional slices of 2D X-ray patterns at 30 m from the preparation during diastole (Dia) and at the force peak (T_p) of a FE or LC twitch, showing the first orders of sarcomeric reflections. Total exposure time 20 ms for Dia, 10 ms for FE and LC. ***B.*** Meridional slices of patterns collected at 1.6 m from the preparation (4 trabeculae), showing the myosin based axial reflections (M1-M6). Total exposure time 150 ms for Dia, 60 ms for FE, 90 ms for LC. ***C.*** Superimposed intensity profiles from A, starting from the 2nd order reflection. ***D.*** Superimposed intensity profiles from B. At Tp all the reflections are weaker, due to the myosin motors moving away from their helical tracks as thick filament switches on.

Results. During force development in a FE twitch sarcomeres shorten against the end compliance so that at T_p SL is reduced from the starting diastolic value, 2.21±0.02 μm, to 1.9±0.03 μm. In the subsequent LC twitch the shortening is largely prevented by a feedforward signal proportional to the shortening in the preceding FE twitch, so that, at T_p, SL is 2.09±0.01 μm and T_p is twice the FE value. We find that in diastole, as in the resting skeletal muscle, all the myosin-based reflections mark the quasi-helical three-stranded symmetry followed by the myosin molecules when they lie in their off state on the surface of the thick filament with a short periodicity (Linari et al. 2015). At T_p the intensities of all the meridional reflections decrease, due to the myosin motors leaving their helical tracks, as the thick filament switches on. The M3 reflection exhibits a different fine structure and different increase in spacing depending on whether the contraction occurs in FE or in LC. On the basis of the structural model of the sarcomere defined for the skeletal muscle (Reconditi et al. 2011), the difference in M3 intensity profiles indicates that during a cardiac twitch only a fraction of motors leaves the off state and this fraction depends on the level of the force independently of the diastolic SL. This work, published in PNAS (Reconditi et al. 2017), provides a quite new integrated view of the Frank-Starling mechanism: independent of the end-diastolic SL (end-diastolic volume), a stress-dependent thick filament regulation adjusts the energetic cost of the heart beat to the ventricular end-systolic pressure-volume relation.

In contradiction with these conclusions a sarcomere length sensitivity of the thick filament structure has been reported (Farman et al. 2011; Ait Mou et al. 2016). To clarify the question, in the second experiment, X-ray diffraction patterns in diastole have been collected in the SL range 1.95-2.25 μm. The intensities of the meridional reflections have been corrected for the effects of the change of diffracting mass in the X-ray beam with SL. We find 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 SL, while their intensities increase with the increase of SL. A similar intensity increase is shown by the first order myosin layer line (ML1), originating from the three stranded helical symmetry of myosin motors on the surface of the thick filament. These results indicate that the order of the quasi-helical three-stranded symmetry of the myosin molecules marking the resting structure of the thick filament in diastole is enhanced with the increase in SL, giving further evidence to the view that the sarcomere length sensitivity modulating the systolic mechanical performance of the heart is not based on the thick filament structure in diastole.

References. Ait-Mou *et al. Proc Natl Acad Sci USA* **113**:2306–11, 2016; Caremani *et al. Proc Natl Acad Sci USA* **113**:3675-80, 2016; Farman *et al. Am J Physiol Heart Circ Physiol* **300**:H2155-60, 2011; Linari *et al. Nature* **528:**276-9, 2015; Reconditi *et al. Proc. Natl. Acad. Sci. U.S.A.* **108**:7236–40, 2011; Reconditi *et al. Proc Natl Acad Sci USA* 2017, doi:10.1073/pnas.1619484114.