ESRF	**Experiment title:** Structural basis of cardiac muscle regulation	**Experiment number**: LS 2570
Beamline: ID02	**Date of experiment**: from: 24 Oct 2016 to: 01 Nov 2016	**Date of report**: 28/02/2017
Shifts: 15	**Local contact(s)**: Theyencheri Narayanan	*Received at ESRF:*

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Report:

The aim of this project was to study thick filament regulation of cardiac contractility in electrically paced intact trabeculae dissected from the right ventricle of rat hearts. We recorded the structural changes in the thick filament structure during a single heartbeat with high spatial and temporal resolution by exploiting the refurbished ID02 beamline and its pool of available detectors.

Methods. Rats were sacrificed by cervical dislocation after sedation with Isoflurane (in compliance with the Home Office Schedule 1) and the heart was rapidly excised and cannulated via the ascending aorta and retrogradely perfused with Krebs-Henseleit solution saturated with oxicarb (95% O_2, 5% CO_2) to have a constant pH=7.4. The sacrifice of the animals was performed at ID17 and the perfused heart was brought to and dissected in the wet lab close to ID02. Trabeculae were dissected under a stereomicroscope and suitable right ventricular trabeculae were mounted in an experimental trough filled with the same solution between the levers of a force transducer and a motor. The solution was continuously exchanged through the trough via a laminar flux between two opposite apertures parallel to the transducer levers. Temperature was continuously monitored and kept constant by controlling the temperature and the flux of the incoming solution. The trough was closed with a cover and sealed with silicon grease and was mounted vertically at the beamline to obtain the best spatial resolution on the meridional axis (parallel to the longitudinal muscle axis). Two mica windows placed as closed as possible to the muscle reduced the X-ray path in water. Platinum stimulating electrodes were positioned along the length of the trabecula.

Results. Intact rat cardiac trabeculae were stimulated at 27ºC at 1Hz; under these conditions the sample could be constantly paced for hours. The sample was moved vertically between X-ray exposures to spread the radiation damage (beam dimension on the sample ~300x220 μm^2, HxV; flux ~$6*10^{12}$ph/s). The total exposure for each sample was ~500 ms. Under these conditions we exploited the flexibility of the ID02 beamline, both in terms of detector pool and range of camera lengths (0.6m to 31m from the sample position). We recorded high-spatial resolution X-ray diffraction patterns with the FReLoN detector during diastole and systole at 31 m camera length to calibrate sarcomere length in each sample from the reflections arising from the sarcomere periodicities. When the detector was moved to 1.6 m camera length we collected the meridional myosin-based reflections (M1, …, M6; Fig. 1A) and the first myosin layer line (ML1, Fig.

1B), orders of a fundamental periodicity of ca. 43 nm. Force generation during systole was accompanied by a decrease in the intensity and an increase in the spacing of the M3 reflection from the regular repeat of the myosin motors along the filament, an increase in the spacing of the M6 reflection, associated with thick filament periodicity, and the loss of the first myosin layer line ML1, associated with the helical order of the myosin motors on the thick filament surface. These changes are similar to those observed during contraction of skeletal muscle.

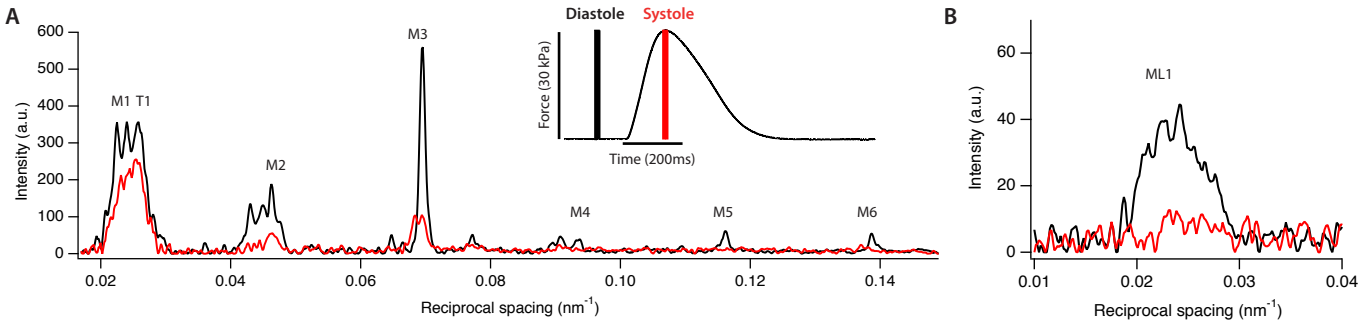

Fig. 1 Meridional intensity profiles (**A**) and intensity profiles of the first myosin layer line reflection (**B**, ML1) from patterns collected during diastole (black trace) and systole (red trace, see inset). FReLoN detector at 1.6m camera length. Data from one trabecula; exposure time, 40ms. Temperature, 27°C; stimulation frequency, 1 Hz.

In the same electrically paced intact cardiac trabeculae it was possible to record time-resolved changes in the X-ray pattern during a single cardiac twitch by using the Pilatus 300k detector to collect 37 20ms-frames (3ms readout; beam attenuated to have 20ms total exposure at each point along the trabecula). The 31m camera length allowed us to measure time-resolved changes in sarcomere length (Fig. 2A) during a single cardiac cycle. With a camera length of 3.2m we were able to acquire for the first time the changes in ML1 (Fig. 2B) and in the meridional reflections up to the M6 during a single heartbeat with 20ms time resolution. These results show that it is possible to describe the kinetics of the OFF/ON transition in the myosin-containing thick filaments during a single heartbeat and reveal the differences with respect to the kinetics of force development and relaxation.

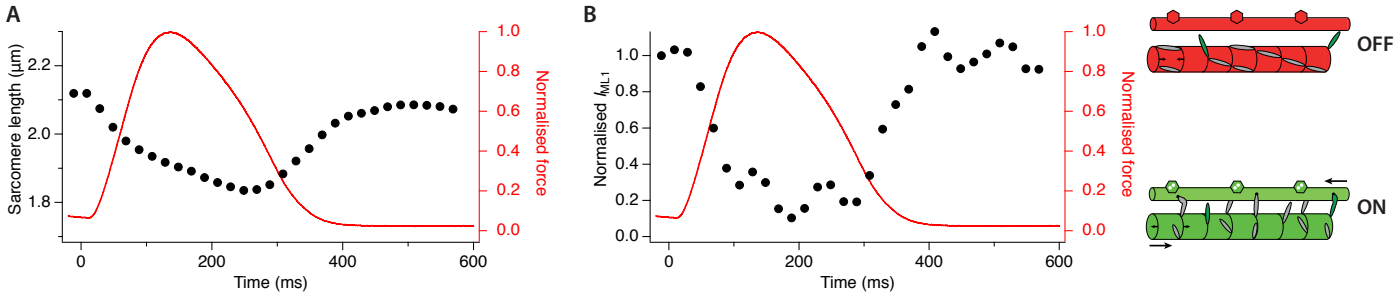

Fig. 2 Time course of the changes in sarcomere length (**A**) and in the intensity of the first myosin layer line reflection (**B**) were measured with the Pilatus detector during a single heartbeat with 20ms time resolution. Pilatus detector at 31m (A) and 3.2m (B) camera length. Data added from three trabeculae; total exposure time per frame, 27.5 ms. Force is the continuous red line. Zero is the time of the stimulus. Temperature, 27°C; stimulation frequency, 1 Hz.

Conclusion. For the first time we showed in a single heartbeat distinct time courses of force, sarcomere length and the X-ray reflections associated with thick filament structure during activation and relaxation, providing new insights into the thick filament regulation of cardiac contractility.