ESRF	**Experiment title:** Calcium dependence of structural changes in the cardiac myofilaments	**Experiment number**: LS 2644
Beamline: ID02	**Date of experiment**: from: 26 Apr 2017 to: 01 May 2017	**Date of report**: 05/09/2017
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. The electrical stimulus triggers a transient increase of intracellular calcium concentration, binding of calcium to troponin in the actin-containing thin filaments, and a structural change in the thin filaments that allows myosin motors from the thick filaments to bind to actin and generate force. We recorded the structural changes in the thin and thick filament structure during a single heartbeat with high spatial and temporal resolution by exploiting the refurbished ID02 beamline and the time-resolved detector Pilatus 300k.

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 ~$5*10^{11}$ph/s). To collect X-ray patterns during the whole cardiac heartbeat, the beam intensity was attenuated to 3% using an iron attenuator

and in a single record the trabecula was exposed for 742 ms (corresponding to 22.3 ms of full-beam exposure). The total full-beam exposure time for each sample was 380 ± 160 ms (mean ± SD, n = 6). Under these conditions we exploited the flexibility of the ID02 beamline in terms of range of camera lengths (3.2m to 31m from the sample position) and the time-resolved detector Pilatus 300k. We recorded X-ray diffraction patterns during a single heartbeat at 31 m camera length to record the sarcomere length changes in each sample from the reflections arising from the sarcomere periodicities. When the detector was moved to 3.2 m camera length we collected the first myosin layer line (ML1) and the meridional myosin-based reflections (M1, …, M6), orders of a fundamental periodicity of ca. 43 nm. Force generation during systole was accompanied by a decrease in the sarcomere length and the loss of the intensity of the first myosin layer line ML1, associated with the helical order of the myosin motors on the thick filament surface (I_{ML1}) with a time course similar to that of force. During the isometric phase of relaxation, characterised by a negligible change in sarcomere length, I_{ML1} was nearly constant. During the so-called chaotic phase of relaxation both sarcomere length and I_{ML1} nearly recovered their diastolic value with a kinetics slower than that of force. On the other hand, the spacing of the M6 reflection (S_{M6}), associated with the thick filament backbone periodicity, had kinetics similar to that of force during both force development and relaxation.

Conclusion. For the first time we described the kinetics of the structural changes in the cardiac thick filament during a single heartbeat. These data provide new insights into the thick filament-based regulation of cardiac contractility.