ESRF	**Experiment title:** Structural dynamics of the thick filament during the physiological working cycle of the heart	**Experiment number**: LS 2864
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Report:

The aim of this work was to study thick filament regulation of cardiac contractility in electrically paced intact trabeculae dissected from the right ventricle of rat heart. We have recently used synchrotron small-angle X-ray diffraction at ID02 to determine the structural dynamics of local domains of the myosin filament during the unitary mechanical response elicited by electrical stimulation of an intact cardiac trabecula at constant length (Brunello E., Fusi L., Ghisleni A., Park-Holohan S.J., Ovejero J.G., Narayanan T., Irving M., *Myosin filament-based regulation of the dynamics of contraction in heart muscle*, PNAS, in press). In those experiments the sarcomeres shortened as force developed, as a result of large compliance at the ends of these isolated heart muscle preparations. This does not happen in the beating heart, which first develops force and pressure at constant volume and sarcomere length, then ejects the blood by sarcomere shortening at roughly constant load. In the protocol used in LS2864, we were able to reproduce that mechanical protocol and separate for the first time the structural changes in the myosin filaments associated with isovolumetric contraction from the later changes associated with ejection. Moreover we were able to determine how those structural changes depend on the load on the heart muscle during ejection, mimicking the response of the intact heart to changes in arterial blood pressure that occur for example in hypertension.

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% O2, 5% CO2) 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 27°C 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 cycle of contraction and relaxation, the beam intensity was attenuated to 3% using an iron attenuator and the trabecula was exposed for 742 ms (corresponding to 22.3 ms of full-beam exposure) in each cycle. The total full-beam exposure time for each sample was 430 ± 110 ms (mean ± SD, n = 8). 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.
The physiological contraction of the heart is divided into four different phases: in the first phase the ventricle develops force at a constant volume (isovolumetric contraction), then in the second phase it contracts at a constant force decreasing its volume to eject the blood; in the third phase the ventricular force decreases at constant volume (isovolumetric relaxation) and in the final phase the ventricle re-expands at low force. We mimicked the working cycle of the heart at the cellular level via a combination of force- and length-clamp protocols in electrically-paced trabeculae. The protocol was optimised by direct measurement of changes in sarcomere length in the same protocols. This is uniquely feasible at ID02 as a result of the beamline optics and possibility to change the sample-to-detector distance in the course of an extended experiment on each sample.

With a sample-to-detector distance of 3.2m we recorded the time course of the changes in the thick filament backbone structure (M6), in the helical order of the myosin motors on the thick filament surface (ML1), in the forbidden reflections (M2 and M5) and the motion of the myosin motors (M3), during the cardiac cycle. We are currently analysing and modeling the data for detailed mechanistic interpretation.

Conclusions. For the first time we described the kinetics of the structural changes in the cardiac thick filament during the physiological cardiac cycle mimicked at the cellular level. These data provide new insights into the thick filament-based regulation of cardiac contractility.