ESRF	**Experiment title:** Molecular basis of length-dependent activation in cardiac muscle	**Experiment number**: LS3078
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Shifts: 12	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

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Report:

The efficiency of the heart as a pump depends on an auto-regulatory mechanism, called Frank-Starling law of the heart, that potentiates the strength of contraction in response to an increase in ventricular filling. At the cellular level this length-dependent activation (LDA) consists of an increase in the sensitivity of the myofilaments to calcium in response to the stretch of the cardiac muscle cell, but the molecular mechanisms which underlie this phenomenon are still unclear. It has been recently hypothesised that LDA is mediated by regulatory structural changes in thin and thick filaments triggered by the passive tension generated at longer sarcomere length by the titin molecule, which runs along the thick filament backbone and connects the thick filaments to the extremities of the sarcomere (Z-bands)[2,3]. However conflicting results have been reported[4-6], suggesting that the stronger contraction of heart muscle at longer lengths is due to a calcium-dependent signalling pathway, possibly involving other regulatory proteins of the thick filament such as myosin binding protein-C, that links stress-sensing in the thick filament with calcium activation in the thin filaments[6].

During LS3078 we exploited the new combination of beam size and brilliance, optics and high sensitivity, time- and spatial-resolution X-ray detector Eiger 2-4M available at ID02 to investigate the structural changes in the myofilaments induced by stretch in relaxed and partially calcium-activated demembranated cardiac trabeculae isolated from rat hearts in near-physiological conditions[7] (27°C and physiological interfilament lattice spacing). The demembranated trabeculae were mounted vertically at the beamline in a custom-made trough at ~2.0 μm sarcomere length. Three 5ms-frames were collected at each calcium concentration at the steady state at the initial fibre length (L_0), just at the end of a 10% L_0 ramp stretch complete in 5ms, and 1s after the ramp, when the viscoelastic mechanical response that follows the stretch had relaxed. We used calcium concentrations in the physiological calcium range (pCa (=$-\log_{10}[Ca^{2+}]$) = 7 to 6).

The Eiger2-4M detector was first placed at 31m sample-to-detector distance for trabecular alignment, protocol optimisation and direct measurement of the first order of the sarcomere length repeat. Sarcomere length measurements are indispensable for averaging X-ray signals from different trabeculae. The detector was then moved to 3.2m sample-to-detector distance to record the X-ray reflections associated with the myosin-containing thick filaments. To reduce X-ray damage, at 31m the beam intensity was reduced with a 50-µm rhodium attenuator, while full-beam was used at the shorter camera length to get enough intensity on the weaker reflections. The data collection period was limited by using a fast shutter, and the trabecula was moved along its long axis between exposures. On each point on the trabecula, 3 5-ms time frames were collected, resulting in a total exposure time per repeat of 15ms. Each trabecula lasted 150-300ms with exposures to the full beam and mechanics and X-ray patterns changed significantly when radiation damage occurred, so that it is easy to discriminate when to stop the experiment.

Preliminary data analysis showed that the data collected are of very high quality and that we can measure changes in the sarcomere repeats, in the equatorial reflections, and in the M3 meridional reflection in each trabecula. Data from weaker reflections need averaging between samples selected on the basis of the behaviour of the X-ray signals analysed in each sample. We will measure the changes in the periodicity of the thick filament backbone associated with the stretch (measured by the spacing of the M6 meridional reflection) and its [Ca^{2+}] dependence. Further analysis and modeling are also in progress to analyse the sub-peaks of the M3 reflection and extract the behaviour of local domains of the myosin filament during this protocol, and to determine if there are any changes in the helical order of the myosin motors on the surface of the thick filament backbone characteristic of the low calcium condition, pCa 9, and measured by the intensity of the first myosin layer line, ML1.

The results of these experiments will help us understand the molecular mechanism underlying LDA in cardiac muscle and in particular what is the role of physiological calcium concentrations on the response to stretch of cardiomyocytes in the physiological range of sarcomere lengths. As LDA is modified in cardiomyopathies, the present results will lead to a better understanding of disease mechanisms, and better design of potential therapies and assays for such therapies.

References

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