ESRF	**Experiment title:** Effect of myosin-targeting drugs on the structural dynamics of the thick filament during the heartbeat	**Experiment number**: LS3040
Beamline: ID02	**Date of experiment**: from: 12/11/2021 to: 16/11/2021	**Date of report**:
Shifts: 12	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

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Report:

Contraction of cardiac muscle on a beat-to-beat basis is triggered by calcium-dependent structural changes in the actin filament controlling the availability of binding sites for the myosin motors, while myosin-based regulation controls the number of avaliable motors and kinetics of force develpment. We have previously exploited the time-resolved USAXS and SAXS at beamline ID02 to determine the structure, function and dynamics of local domains of the myosin filament during the unitary mechanical response elicited by electrical stimulation of intact cardiac trabeculae at a constant length with 20ms time resolution (Brunello et al., *PNAS* 117:8177-8186, 2020).

Here we further tested the effect of calcium *per se* on the activation of the cardiac thick filament when force development is prevented by shortening against low load. We used small-angle X-ray diffraction from intact trabeculae isolated from rat hearts, electrically paced at 1Hz and constantly perfused with Kreb's buffer at 27°C, a temperature that preserves myosin-based regulation in intact cardiac muscle (Ovejero et al. *J Gen Physiol*, 2022).

The high-time and high-spatial resolution detector Eiger2-4M 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, the beam intensity was reduced with a 50-µm rhodium attenuator, 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, 38 20-ms time frames were collected, resulting in a total exposure time per beat of 762ms.

Preliminary data analysis showed that shortening against low load applied just after the electrical stimulus prevents force development and the changes in the periodicity of the thick filament backbone (measured by the spacing of the M6 meridional reflection). Further analysis and modeling are 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 relaxed phase of the heatbeat (measured by the intensity of the first myosin layer line ML1).

These results of these experiments will consolidate the concept of myosin-based regulation in cardiac muscle, and support our previous findings in skeletal muscle (Linari et al. Nature 2015). A better understanding of thick-filament regulation of cardiac contractility will help devise better tests for myosin-targeting drugs, and potential therapies and assays for such therapies.