ESRF	**Experiment title:** Time course of the changes in thick filament structure during the relaxation phase of the heartbeat	**Experiment number**: LS2987
Beamline: ID02	**Date of experiment**: from: 07/07/2021 to: 12/07/2021	**Date of report**:
Shifts: 15	**Local contact(s)**: Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Luca Fusi, King's College London

*Dr. Yanhong Wang, King's College London

*Dr. Cameron Hill, King's College London

Dr. Elisabetta Brunello, King's College London

Prof. Malcolm Irving, King's College London

Dr. Zaibaa Patel, King's College London

Dr. Ivanka Sevreiva, King's College London

Report:

The pumping action of the heart is driven by rhythmic contractions of its muscular walls. We previously exploited the characteristics of beamline ID02 to determine the kinetics of the structural changes in the cardiac thick filament during the unitary mechanical response elicited by electrical stimulation of an intact cardiac trabecula at constant length (Brunello et al., *PNAS* **117**:8177-8186, 2020).

Here we characterised the structural changes in the cardiac thick filament during the physiological working cycle of the heart, by applying mechanical protocols that can mimick at the cellular level the four phases of the cardiac cycle: ventricular filling, isovolumetric contraction, ejection at constant force and isovolumetric relaxation. We used X-ray diffraction from intact trabeculae isolated from rat hearts and constantly perfused with Kreb's buffer at 27°C, a temperature at which the structure of the thick filament is similar to that at physiological temperature (LS2788; manuscript in preparation).

For each trabecula, the high-spatial and time-resolution detector Eiger2-4M was placed at 31m camera length to optimise the mechanical protocols by direct measurement of the first order of the sarcomere length repeat. The detector was then positioned at 4.4m camera length to allow recording of the X-ray reflections associated with the myosin-containing filaments. The X-ray beam was small (FWHM, 150 μm x 26 μm, HxV) in order to interrogate only the sarcomeres in a coherent region of the sample. X-ray acquisition consisted of 38-frames with 20-ms time resolution resulting in a total exposure time per cardiac cycle of 762ms per point on the trabecula, therefore beam was attenuated to reduce radiation damage on the sample.

Preliminary analysis of the data showed that during the isovolumetric phase of contraction the activation of the thick filament (measured by the increase in the spacing of the filament backbone S_{M6} and decrease in the intensity of the first myosin layer line indicating disruption of the folded conformation of the myosin motors on the thick filament surface) is faster then during activation at constant trabecular length, but reaches the

same value at the same peak force. In addition, we found that the thick filament inactivates rapidly during the isometric relaxation phase of the cardiac cycle. Further analysis and modeling are in progress to describe the structure, dynamics and function of the myosin motor domains in different regions of the thick filament during the cardiac cycle.

These results show for the first time that the cardiac cycle can be accurately mimicked at the cellular level in a preparation in which the molecular structural changes that drive the cycle can be determined by time-resolved X-ray diffraction and interference. The results will elucidate the fundamental molecular mechanisms that drive and regulate the cycle. They also establish an assay that can be used in future studies to test the effect on cardiac function of point mutations in sarcomeric proteins or of drugs that target myofilament proteins.