ESRF	**Experiment title:** Myosin-based regulation of contractility in the heart: characterisation of the ON structure of the myosin filament induced by cooling	**Experiment number**: LS 2788
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Shifts: 15	**Local contact(s)**: Theyencheri Narayanan	*Received at ESRF:*

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Report:

The emerging concept of a dual-filament mechanism of contractile regulation has focused attention on the factors including mechano-sensing and RLC phosphorylation that can alter the regulatory state of the thick filament. However the structural basis of thick filament regulation is still poorly understood.

Here we characterised the structural basis of the regulatory transition in the cardiac thick filament induced by cooling from 37ºC to 9ºC, using X-ray diffraction from intact and demembranated trabeculae isolated from rat hearts. Thick filament structure in skinned trabeculae in relaxing solution in the presence of 3% dextran T500 at 27-37°C was similar to that in intact quiescent trabeculae. Maximal calcium activation at 27°C was used as a reference for the ON state of the thick filament.

Cooling of intact quiescent trabeculae induces a decrease in the intensity of the meridional myosin-based reflections and of the first myosin layer line indicating disruption of the OFF state of the thick filament. This was associated with an increase in the spacing of the M6 reflection (S_{M6}) associated with the axial periodicity of the thick filament backbone, a biphasic change in that of the M3 reflection (S_{M3}) associated with the myosin motors, and an increase in the equatorial intensity ratio (I_{11}/I_{10}) associated with the movement of myosin motors towards the thin filaments. Cooling of demembranated trabeculae in relaxing solution showed larger changes in the intensity and spacing of the myosin-based reflections, and S_{M3}, S_{M6} and I_{11}/I_{10} in relaxing solution at 9ºC were close to their respective ON values.

The results show that cooling intact quiescent or relaxed skinned trabeculae induces a transition in the structure of the thick filament from the OFF towards the ON state in the absence of calcium activation and filament stress. The structural changes are smaller in intact than skinned trabeculae.