Molecular mechanism of regulation of muscle contraction (SC-3245, Nov 2011)

The aim of this project was to elucidate the conformation of the myosin heads in the detached state during an isometric contraction to further constrain the structural model derived by combined mechanical and diffraction experiments on muscle fibres of the frog and to investigate whether and how resting links between myosin and actin filaments may influence the filament structure and contribute to regulation of muscle activation (see Proposal SC-3245). For this purpose we have recorded X-ray diffraction patterns from small bundles of muscle fibres (2-3 fibres per bundle) of *Rana esculenta* at different sarcomere length (*sl*) in the range 2.1 to 3.6 μm, both at rest and during an isometric contraction. The experiments have been repeated at two different camera lengths: at 6 m to record the fine structure of the inner reflections (such as the MyBPC-based M1 at ca 42-44 nm, the troponin-based T1 at 38 nm, the myosin-based M3 at 14.5 nm associated with the axial repeat of the myosin heads) and the reflections associated with the periodicity of the sarcomeres, and at 2.2 m to record the higher order myosin-based M6, associated with the periodicity of the backbone of the thick filament, and the actin-based layer lines at 5.1 and 5.9 nm.

Results. The experiments have shown that the intensity of the (1,0) equatorial reflection, $I_{1,0}$, at each *sl* in the resting fiber is independent of the *sl*, once normalised for the cross sectional area of the sample and for the different number of sarcomeres illuminated by the beam at different *sl*. A precise normalization for the diffracting mass in the X-ray beam can thus be achieved by dividing each pattern by the corresponding $I_{1,0}$ at rest.

The very low background scatter of the ID02 camera allowed us to record the intensities of meridional reflections very close to the beam-stop that correspond to orders of the half-sarcomere length[1] and therefore allow the *sl* in the local region of the fiber illuminated by the X-ray beam to be determined directly from the X-ray data.

In resting fibers, the intensity of both the 44-nm meridional reflection, M1, associated with myosin and myosin-binding protein C (MyBPC) and the first myosin layer line, ML1, associated with the helical order of myosin heads decreased in the *sl* range 2.6 to 3.0 μm but were constant outside it (Fig. 1). The spacing of both the M3, S_{M3}, and M6, S_{M6}, reflections are almost constant up to *sl* ~ 2.7 μm and then start to increase at longer *sl*.

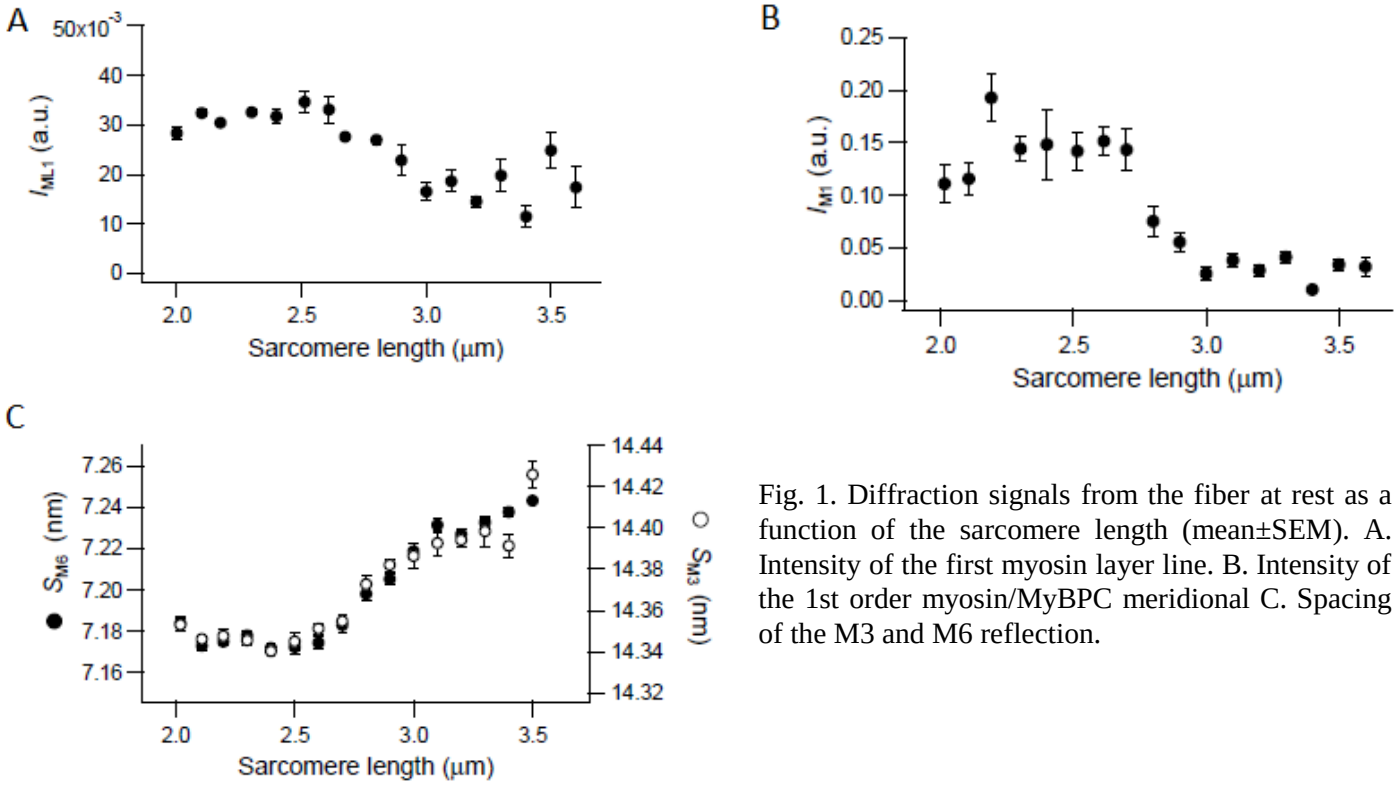

Fig. 1. Diffraction signals from the fiber at rest as a function of the sarcomere length (mean±SEM). A. Intensity of the first myosin layer line. B. Intensity of the 1st order myosin/MyBPC meridional C. Spacing of the M3 and M6 reflection.

The observed structural changes for *sl* 2.5-3.0 μm occur in the same *sl* range where the overlap between MyBPC, confined to the 'C-zones' of the thick filament from 250 to 510 nm from the filament midpoint[2], and actin decreases. This suggests that the OFF conformation of the thick filament is maintained by an

interaction between the MyBPC-containing region of the resting thick filament with overlapping thin filaments, providing a clear indication of structural and functional links between myosin and actin filaments via MyBPC.

In the *sl* range 2.0 to 2.9 μm, the intensity of the M3 reflection, I_{M3}, during isometric contraction, like force, is directly proportional to the degree of overlap between thick and thin filaments. For *sl* > 2.9 μm the reflection becomes weak and more difficult to measure. This could be due to dynamic instability between sarcomeres that can affect both I_{M3} and the interference fine structure of the M3 reflection (R_{M3}). For these reasons we limited detailed analysis and interpretation of the sarcomere-length dependence of I_{M3} and R_{M3} to the range 2.0 to 2.9 μm (Fig. 2). The result that during active contraction I_{M3} is proportional to the degree of overlap between thick and thin filaments suggests that the myosin heads in the non-overlap region of the thick filament are axially disordered and make little or no contribution to the intensity of the M3 reflection, which is dominated by the contribution of the actin bound-heads in the overlap region. We used the I_{M3} and R_{M3} data to test the prediction of previously proposed models for the conformation of the detached myosin heads and found that the model with one population of detached heads[3] fails to reproduce the data, while the model with two different populations of detached heads[4] predicts a sarcomere length-dependence of both I_{M3} and R_{M3} in agreement with the experimental data (Fig. 2).

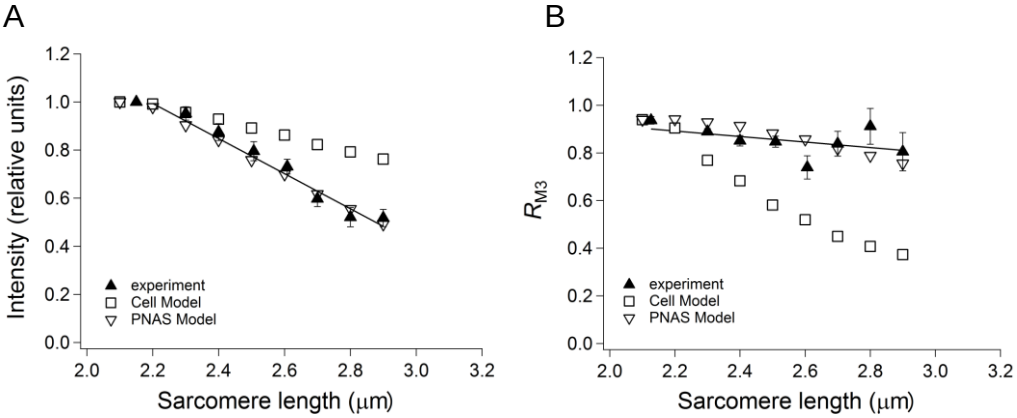

Fig. 2. Sarcomere-length dependence of the M3 reflection during active isometric contraction. A. Intensity and (B.) ratio (R_{M3}) of the higher and lower angle peaks of the M3 reflection. Black triangles: experimental data. Lines: linear regression on data. Squares and open triangles are the prediction of the models described in [3] and [4] respectively

References:[1]Bordas et al. (1987) J. Cell Biol. **105**: 1311-1318.; [2]Luther et al. (2011) *PNAS* **108**:11423; [3]Piazzesi et al. (2007) *Cell* **131**:784; [4]Brunello et al. (2007) *PNAS* **104**:20114