

Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

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Primary CNF Tools Used: Primary tools: GCA 5X Stepper, SUSS MA6-BA6 Contact Aligner, Photolith spinners, Oxford 81 ion etcher, Reynolds Tech KOH Hood

Abstract:

The production of custom silicon-nitride force nano-levers allows for the measurement of nanometre-level forces in individual myofibrils [1]. Using the optical displacement of cantilevers attached to individual myofibrils or isolated sarcomeres permits the quantification of mechanical parameters associated with basic muscle properties (Figure 1). Recently, our lab has used cantilever-based force measurements in isolated myofibrils to investigate the behaviour of the sarcomeric Z-line under stress, and the behaviour of the gigantic spring-like protein titin. Briefly, for the first time, we have established that the sarcomeric Z-line may undergo extreme and reversible expansion at extreme sarcomere lengths (Figure 2). Notably, this expansion appears to be restricted to sarcomeres from type II muscle fibres and may constitute an injury prevention mechanism in skeletal muscle at extreme sarcomere lengths (Figure 2). Second, following our recent advances in immunolabeling techniques of titin (see [2]), we have mechanically quantified segmental extension of titin in passive myofibrils. This has led to a couple of distinctive insights: first, the distal immunoglobulin domains of titin appear to be extensible when titin is in its in situ sarcomeric configuration (Figure 3); second, that at long sarcomere lengths ($>3.8\mu\text{m}/\text{sarcomere}$), we confirmed that titin does indeed pull out of the A-band (Figure 3). Last, in collaboration with the High-Resolution Macromolecular Imaging Facility at the University of British Columbia, we have developed a unique system that allows for imaging of individual sarcomeres and myofibrils using cryo-electron tomography (Cryo-ET) after mechanical testing is performed on those exact same myofibrils (Figure 4). This system will allow for the correlation of different mechanical states of sarcomeres with angstrom-level resolution of protein structures within those same sarcomeres and therefore has the potential to provide significant insight into the

molecular mechanisms of muscle function. Importantly, all recent advancements have been made possible by the use of the custom silicon-nitride force nano-levers that allow for measurements of force production in individual sarcomeres and myofibrils.



Figure 1: Example myofibril held between a glass needle and force nanolevers imaged under phase contrast.

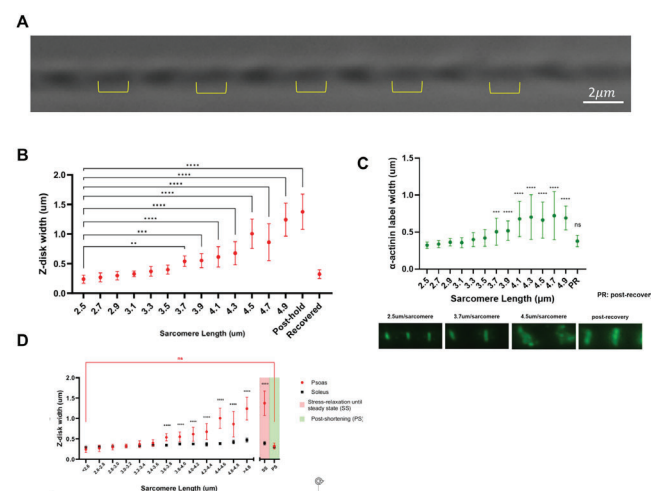


Figure 2: (A) Example myofibril held at $\sim 4.5\mu\text{m}/\text{sarcomere}$ showing significant apparent Z-line expansion. (B) Significant Z-line expansion occurs in rabbit psoas myofibrils at a length $\geq 3.7\mu\text{m}/\text{sarcomere}$ ($n \geq 8$ sarcomeres per point, one-way ANOVA, Dunnett's multiple comparisons test). (C) α -actinin label width as a function of sarcomere length shows significant expansion also at lengths $\geq 3.7\mu\text{m}/\text{sarcomere}$ that appears recoverable after a slack period of 10 min ($n \geq 30$ sarcomeres per point, one-way ANOVA, Dunnett's multiple comparisons test). (D) Z-line expansion is type II fibre-specific and is absent in soleus (type I).

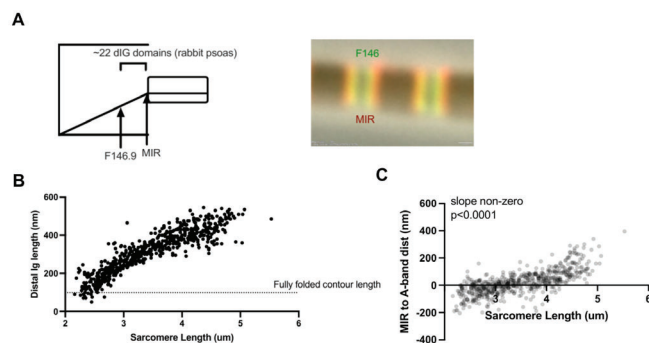


Figure 3: (A) Location of distal PEVK (F146.9) immunolabel and A-band insertion label (TTN-MIR), visualized with AF488 and AF647 secondary antibodies, respectively. (B) Distal Ig domains clearly demonstrate extension past fully folded contour length indicating unfolding of Ig domains occurs. (D) Location of TTN-MIR immunolabel relative to the A-band shows a significant non-zero slope indicating titin is pulled out of the A-band at long (>3.8 μm/sarcomere) sarcomere lengths ($P<0.001$, F -test).

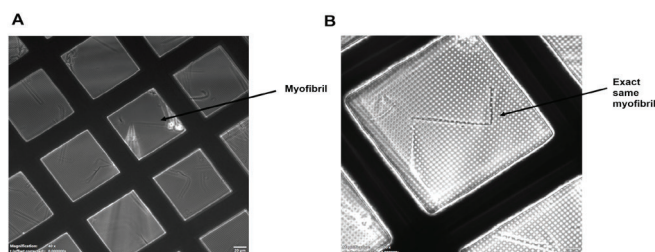


Figure 4: (A) Myofibril attached to mechanical testing apparatus suspended above Cryo-ET grid immediately prior to placement. (B) The same myofibril visualized in A post-placement on the grid and following grid removal for plunge freezing. The myofibril retains its sarcomere length and can be found intact exactly as it was placed on the grid.

Summary of Research:

Imaging of myofibrils was performed on an inverted Olympus IX83 microscope in both phase contrast and fluorescent channels (where applicable). Images were analysed using CellSens® Dimensions software. Myofibrils were attached to a glass needle and custom silicon-nitride force-nanolevers; the glass needle was displaced using a piezoelectric motor, and force was measured using optical displacement of the force nanolevers based on known spring constants.

First, following observations of Z-line expansion at extreme sarcomere lengths in unrelated myofibril testing (Figure 2A), we investigated the behavior of the sarcomeric Z-line in rabbit psoas (type II) myofibrils.

We found significant expansion of the Z-line through both phase contrast imaging (Figure 2B) and using α -actinin immunolabels specific to the Z-line (Figure 2C) at extreme sarcomere lengths. Notably, after a 10 min recovery period at slack length, Z-lines returned to normal width, suggesting that this undescribed phenomenon is reversible. We attempted similar experiments in rabbit soleus (type I) myofibrils and found significantly reduced Z-line expansion, suggesting that this phenomenon is fibre-type specific (Figure 2D).

Second, we placed titin immunolabels at the distal end of titin's PEVK and at titin's main immunogenic region (MIR), located at the A-band insertion of titin (Figure 3A). This allowed for the quantification of extension of the distal immunoglobulin domain extension in isolated myofibrils. Results suggested that these domains were highly extensible (Figure 3B), in opposition to assumptions in the field that they were fixed [3]. We also noted that at long sarcomere lengths >3.8 μm/sarcomere, titin's insertion into the A-band, measured by the MIR immunolabel, pulled out of the A-band (Figure 3C).

Third, we have developed a unique protocol where a myofibril may be tested mechanically using the custom force-nanolevers, then placed directly onto a Cryo-ET grid (Figure 4A) while preserving the mechanical state of the myofibril. Then, the Cryo-ET grid can be removed for immediate plunge freezing, and the same myofibril subsequently identified (Figure 4B) for imaging using Cryo-ET. This method will allow, for the first time, correlation of mechanical properties at the sarcomeric level with protein structures in individual sarcomeres and thus can significantly advance mechanistic understanding of muscle function.

Conclusions and Future Steps:

In conclusion, the use of custom force nanolevers for quantification of myofibrillar forces has been critical to developing our understanding of muscle mechanics. Using immunofluorescent and phase contrast imaging coupled with simultaneous force measurements of myofibrils has permitted the identification of novel phenomena, such as Z-line expansion, and the development of novel techniques such as mechanically coupled Cryo-ET of individual sarcomeres.

References:

- [1] M. E. Fauver, et al. IEEE Trans Biomed Eng 45(7):891-898, 1998. DOI: 10.1109/10.686797
- [2] D. Desai, et al. J Exp Biol 229(2): jeb250931. <https://doi.org/10.1242/jeb.250931>
- [3] P. Bennett, et al. J Struct Biol 120(1): 93-104. <https://doi.org/10.1006/jsbi.1997.3898>