

Specimen Description

Full Sample Name (no acronyms)

Description of Sample (buffer concentrations, additives, etc.): 5 mM MgAcetate, 5 mM NaATP, 5 mM EGTA, 20 mM MOPS, 100 mM NaCl, 5 mM DTT, and 10 mM Na-PO₄ (pH 7.0)

Total Mass (kDa)

$(32,000 \text{ \AA} - 1,600 \text{ \AA})/145 \text{ \AA} = 210 \text{ crowns}$

$210 \times 4 = 840$ myosin molecules	$840 \times 520,000 = 436,800,000$
$210 \times 4 = 840$ flightin molecules	$840 \times 20,000 = 16,800,000$
$210 \times 4 = 840$ myofilin molecules	$840 \times 20,000 = 16,800,000$
$210/5 \times 2 = 84$ paramyosin molecules	$84 \times 214,000 = 17,976,000$
$210/5 \times 4 = 168$ stretchin-klp molecules	$168 \times 231,000 = 38,808,000$

Total mass = 527,184 kDa

Estimated Dimensions: Diameters up to 420 Å, Lengths from 1.6 micrometers to 3.2 micrometers.

Particle Symmetry: C-3 (vertebrate), C-4 (invertebrate), or C-7 (molluscan) depending on filament type

Final Sample BSL: No containment needed. Completely non-pathological.

Figures/Preliminary Data

Our preliminary results are largely from the flight muscles insects that utilize asynchronous flight muscle. These include the Hemipteran *Lethocerus indicus*, and the Dipteran *Drosophila melanogaster*. We plan to investigate vertebrate striated muscle thick filaments as well.

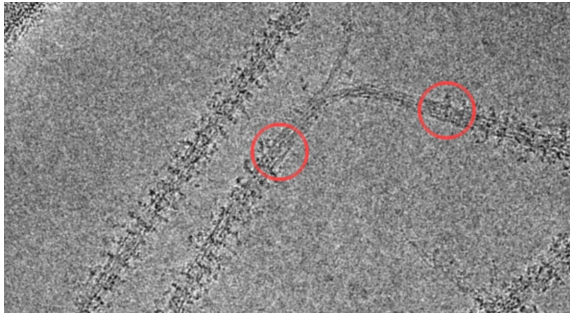


Figure 1. cryoEM of a frozen-hydrated *Lethocerus* thick filament recorded in integration mode on the DE-64. Red circles outline the first crown (crown-0). At the bare zone ends heads become ordered into shelves called “crowns”. Although all parts of the thick filament showing ordered myosin heads are useful for a helical reconstruction, only those for which crown-0 can be identified are useful for the asymmetric reconstruction. If asymmetric reconstruction shows sufficient variability along the filament, potentially multireference alignment can place filament segments in their correct location.

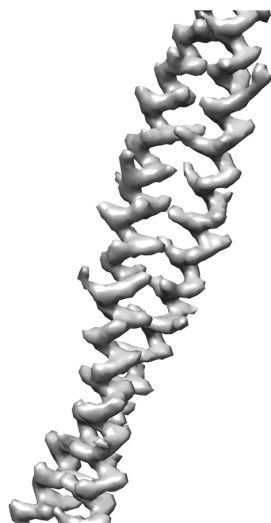


Figure 3. Portion from the 2.8Å reconstruction of flight muscle thick filaments from *Drosophila melanogaster* showing well-resolved side chains. This region from the myosin α -helical coiled coil tail but not the same region shown in Figure 2.

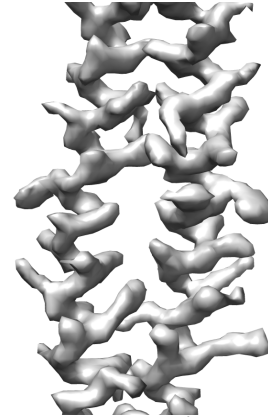


Figure 2. Portion of the 2.7 Å reconstruction (FSC) from *Lethocerus indicus* showing well resolved side chains. This region from the myosin α -helical coiled coil tail.

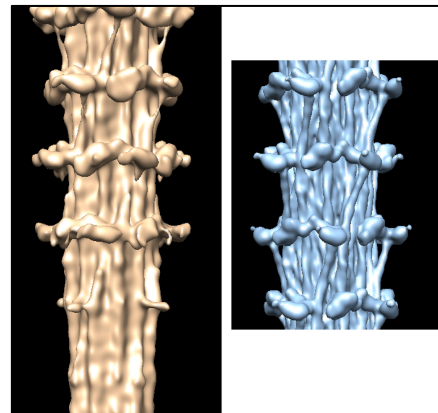


Figure 4. Comparison of *Lethocerus* thick filaments reconstructions. (left) The crown-0 reconstruction which imposed only rotational symmetry on 542 motifs (2,168 asymmetric units), reaching 16Å resolution by FSC criteria. Images were recorded in integration mode on a DE-64 detector. (right) Previously published 6Å helical reconstruction from ~24,000 segments, low pass filtered to 16Å. Images recorded on a DE-20 direct electron detector operated in integration mode. The DE-20 detector is inferior to both the DE-64 and K3. Both reconstructions show myosin heads projecting from the backbone and their S2 linkage, though not as well as in the crown-0 reconstruction.

Bibliography

1. Hu, Z.; Taylor, D. W.; Reedy, M. K.; Edwards, R. J.; Taylor, K. A., Structure of myosin filaments from relaxed *Lethocerus* flight muscle by cryo-EM at 6 Å resolution. *Sci Adv* **2016**, 2, (9), e1600058.
2. Daneshparvar, N.; Taylor, D. W.; O'Leary, T. S.; Rahmani, H.; Abbasiyeganeh, F.; Previs, M. J.; Taylor, K. A., CryoEM structure of *Drosophila* flight muscle thick filaments at 7 Å resolution. *Life Sci Alliance* **2020**, 3, (8), e202000823.
3. Rahmani, H.; Ma, W.; Hu, Z.; Daneshparvar, N.; Taylor, D. W.; McCammon, J. A.; Irving, T. C.; Edwards, R. J.; Taylor, K. A., The myosin II coiled-coil domain atomic structure in its native environment. *Proc Natl Acad Sci U S A* **2021**, 118, (14), e202415111.
4. Abbasi Yeganeh, F.; Rastegarpouyani, H.; Li, J.; Taylor, K. A., Structure of the *Drosophila melanogaster* Flight Muscle Myosin Filament at 4.7 Å Resolution Reveals New Details of Non-Myosin Proteins. *Int J Mol Sci* **2023**, 24, (19), 14936.
5. Li, J.; Rahmani, H.; Abbasi Yeganeh, F.; Rastegarpouyani, H.; Taylor, D. W.; Wood, N. B.; Previs, M. J.; Iwamoto, H.; Taylor, K. A., Structure of the Flight Muscle Thick Filament from the Bumble Bee, *Bombus ignitus*, at 6 Å Resolution. *Int J Mol Sci* **2023**, 24, (1), 377.