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High Resolution Cryo-EM Structure of *Drosophila* Thick Filaments

Fatemeh Abbasi Yeganeh[1], Hosna Rastegarpouyani[1] and Kenneth A. Taylor[1*]

[1.] Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, United States.
* Corresponding author: ktaylor@fsu.edu

Drosophila melanogaster is a highly studied genetic model organism for getting a better understanding mechanism of human disease. Nearly 75% of human disease-causing genes are believed to have a functional homolog in *Drosophila* [1]. *Drosophila* uses indirect flight muscle (IFM), which is stretch activated, to enable wings beating at high frequencies. Stretch-activation is an active force response to stretch, and it is independent of nerve impulses in the muscle as beating rate in wings are much higher than nerve pulses. After discovery of stretch activation using *Lethocerus* [2], indirect flight muscle has been a staple subject of study to gain more understanding into muscle structure and function. *Drosophila* and *Lethocerus* are very similar structurally and *Drosophila* has the benefit of being a model organism which can be genetically manipulated [1].

Sarcomeres of striated muscle are the repeating unit mainly responsible for coordinating muscle contraction. These units are composed of four basic components: bipolar, myosin-containing thick filaments; polar, actin-containing thin filaments; a Z-disk which cross-links antiparallel actin filaments into a bipolar structure; and a connecting filament to link the thick filaments to the Z-disk. Here we are concerned with the myosin-containing thick filaments, the least characterized component structurally.

Despite all the efforts in understanding of thick filaments in muscle, the atomic structure of thick filaments remains unknown. In our group we use cryo-electron microscopy to image isolated, frozen-hydrated specimens which enables us to solve structure of thick filaments in their native state. My project focuses on improving the 7Å resolution structure of *Drosophila* fight muscle thick filament which is already published [3]. *Drosophila* flight muscle gives us the unique opportunity to pursue different mutations. Invertebrate thick filaments show helical symmetry and are much more ordered, longer, and homogeneous compared to vertebrates which makes them a much more suitable subject for cryo-EM.

The previous resolution was limited by different factors, such as larger pixel size due to low magnification imaging and heterogeneity cause by disordered myosin heads. Here I have improved the resolution to 5.7 Å resolution by taking new, high-quality data with better sample preparation. The results show significantly better map compared to 7Å resolution structure.

Striated muscle thick filaments are composed of myosin II and several non-myosin proteins. Myosin II's long α-helical coiled-coil tail forms the dense protein backbone of filaments while its N-terminal globular head containing the catalytic and actin binding activities extends outward from the backbone. Here we report the structure of thick filaments of the flight muscle of the fruit fly *Drosophila melanogaster* at higher resolution of 5.7Å than the previous resolved structure at 7Å which differ significantly from images of *Lethocerus* in the order of the myosin heads and in the non-myosin proteins. The reconstruction shows four densities that cannot be assigned to myosin. Two of those densities are similar to non-myosin densities in *Lethocerus* thick filaments, that we believe them to be the flightin and myofilin, these densities have been resolved with higher resolution that the 7 Å map.

One new density, possibly stretchin-Mlck, is also found on the backbone along with several unexpected features. Stretchin-klp [4], is the form detected in our proteomics. We were able to fit PDB(2YXM) of I-set domains from human myosin binding protein C to these densities Contrary to the generally accepted model for the myosin tail packing into subfilaments, the myosin tails were found organized into curved molecular crystalline layers, referred to here as ribbons, due to their rather flat and narrow but elongated morphology.

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