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Homology model of *Drosophila melanogaster* myosin filaments

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Striated muscle is made of muscle cells or fibers, consisting of hundreds of myofibrils which are usually about 1 to 2 μm in diameter. Myofibrils are composed of repeating units called sarcomeres which are arranged in tandem for the entire length of the muscle and are responsible for generating tension during muscle contraction. Sarcomeres 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. Muscle contraction and force generation is caused by interactions between myosin heads (thick filaments) and actin filaments (thin filaments). Myosin consists of a head which contains the molecular motor and a long, 1600 Å a-helical coiled coil tail which comprises much of the mass of the thick filament backbone. Myosin's tail is its least understood domain. *Drosophila melanogaster*, the fruit fly, is a highly used genetic model organism in the study of human disease. Nearly 75% of human disease-causing genes are believed to have a functional homolog in *Drosophila [1]*. Recent progress in understanding myosin structure was achieved by an atomic resolution cryoEM reconstruction of its complete tail domain within flight muscle thick filaments of the large water bug, *Lethocerus indicus [2]*.

The myosin tail is a highly conserved structure. In *Lethocerus* and *Drosophila*, the tail sequences are 90% identical to each other; their structures within thick filaments, which are arranged in "curved molecular crystalline layers" are also highly similar. In *Lethocerus*, the myosin coiled coil in situ proved to be very similar to crystallized segments of human cardiac b-myosin. Likewise, the *Drosophila* flight muscle myosin tail sequence is 56% identical to human cardiac β-myosin (MYH7). Similar arrangement and high conservation in the myosin tail points at the similarities between *Drosophila* and cardiac myosin filaments. The myosin tail provides more than simply a device for assembly of myosin molecules into filaments. Of the ~500 myosin mutations known to cause muscle disease in humans, ~40% are located in the tail domain [3]. Thus, an atomic model of the *Drosophila* myosin tail can potentially inform on the mechanism in familial human cardiac disease.

Here we introduce a homology model of *Drosophila* myosin filaments built based of atomic model of *Lethocerus* thick filaments. An atomic model will include important information about the formation, arrangement, and interactions between myosin tail which is only observed within intact thick filaments. These interactions can be directly related to skeletal muscle, cardiac muscle, and muscle disease. We have published a structure of *Drosophila* thick filaments at 7Å resolution [4] and our lab has built an atomic model from Lethocerus thick filaments. Although 7Å is not enough resolution to build an atomic model di novo, but we were able to build a homology model based on *Lethocerus*. Similarities between *Lethocerus* sequence and *Drosophila's* sequence makes our homology model more reliable. Also, with our structure despite not being able to see side chains, we are still able to observe key features like coiled coils and their interactions. The *Lethocerus* atomic model was built di novo using COOT [5] and fit in the structure using MDFF. In order to build the atomic model for *Drosophila*, we mutated *Lethocerus* myosin tail sequence to *Drosophila* using COOT and ran a rigid fit into the myosin rod structure. Then we moved to MDFF [6] to fit the homology model into segmented myosin rod. Copies of the myosin tail can be placed in the thick filament backbone following helical and C4 symmetry which will provide us with the full atomic model of *Drosophila* thick filament

backbone. In order to verify our model, we ran the coiled coil through CCCP and detected the four expected skip regions.

This homology model can be used to explain various observations in Drosophila genetic research. For example, we can see the interaction between the myosin tail and Flightin at E1554 which explains why mutating this residue to a Lysine (E1554K) eliminates the *in vivo* incorporation of Flightin [7]. Interaction with other myosin associated proteins can also be investigated to achieve a better understanding of their role in myosin filament. Fly-lines with disease-causing mutations in their myosin heavy chain that is not investigated structurally and our homology model will be a valuable reference to understand them.

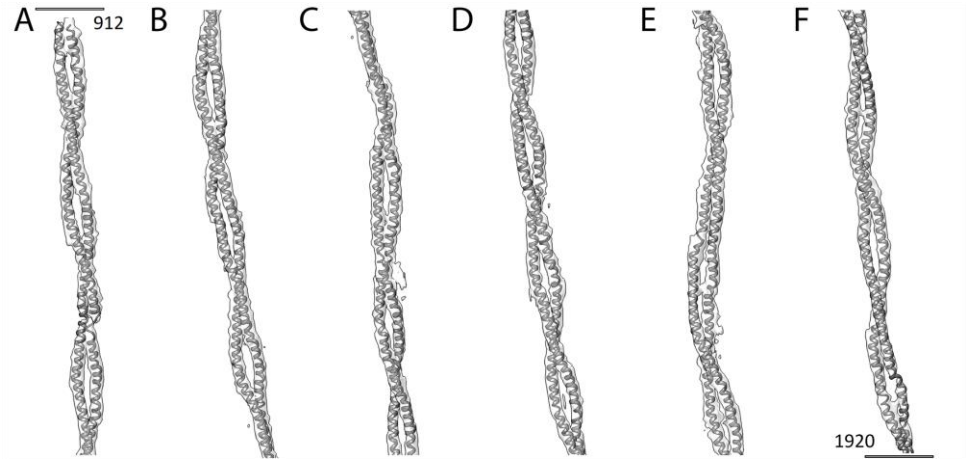

Figure 1. Myosin tail homology model fit in myosin rod structure. Images are sorted in panels A to F from N-terminus towards C-terminus starting at residue 912 and ending with residue 1920.

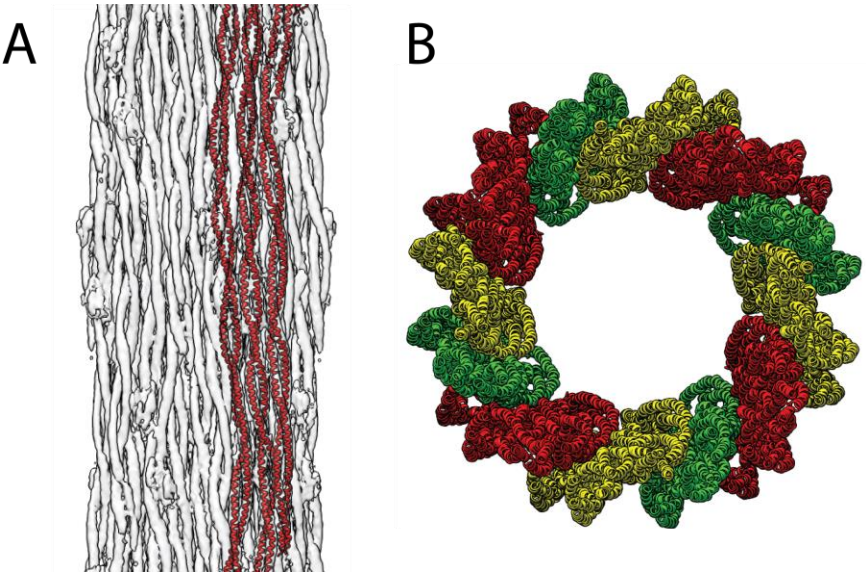

Figure 2. Crystallin layers (ribbons) packing of the backbone. Different ribbons are colored in red, green, and yellow. (A) Atomic model of one ribbon fit to a transparent rendition of the *Drosophila* backbone. (B) Top view of the myosin tails arranged in thick filament backbone.

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