



Fig. 1. Computer-generated representation of myosin VI binding to an actin filament. Actin filament (white) is shown with a myosin VI molecule attached through its globular head domain (teal) with the lever arm (yellow) and tail domains (blue, red, green) shown in three positions as they undergo a 180° swing. Inset shows representation of putative single helix predicted for the structure of MT domain of the tail. Cargo binding domain is not shown.

TAKING BIG STEPS WITH MYOSIN VI

The interaction of muscle myosin (the large family of motor proteins found in eukaryotic tissues) with actin filaments generates the force that powers muscles. In an elegant example of nature's endless ability to adapt old tools to new projects, recent research years has revealed a family of myosin proteins that move along actin filaments in unexpected ways to carry loads within cells. One of these proteins, myosin VI, is of particular interest because its predicted dimensions cannot account for its large (~36-nm) steps. In order to determine whether more information about the structure of myosin VI might uncover the secret of these large steps, researchers at Stanford University used the 12-ID beamline at the APS to study the structure of the tail portion of myosin VI that is predicted to be responsible for step size. Because there are few other natural examples in known protein structures, understanding the role this single helical domain plays in myosin VI force generation may provide a model for the design of synthetic proteins in which a single secondary structure that spans a long distance may be of value, such as in the design of novel drug delivery systems.

Muscle myosin contains a globular head that hydrolyzes adenosine triphosphate (ATP, a high-energy phosphate compound found in the body) to provide the force to ratchet the molecule along actin filaments in a stepwise manner. Association of the elongated myosin tail with oppositely aligned myosins on other actin filaments creates a sliding motion that is typical of skeletal muscle movement.

Using small-angle x-ray scattering (SAXS) data collected at the XOR/BESSRC beamline 12-ID in combination with other techniques, the Stanford researchers obtained evidence that myosin VI takes its big steps by virtue of a rare, single-helical domain. Analysis of the amino acid sequence of myosin VI revealed the predicted globular head region followed by a lever arm, tail region, and cargo binding domain. The tail region is predicted to contain three domains that may be involved in step size; these are called the proximal, medial, and distal tail regions on the basis of their location relative to the myosin head domain.

Working with expression constructs of the tail region, investigators studied the role of these domains. The proximal tail (PT) region, closest to the lever arm and head, has been proposed to form a random coil that may

allow for the flexibility required for the elongated step. Hypothesizing that substitution of amino acids would not disrupt the function of this region if it was a random coil but would if the coil was structured, the team made a series of mutations in the PT and found that these changes significantly affected the step size of the myosin. Their data do not support a model in which step size is facilitated by the elongated random coil structure of the PT, but do show that the PT is likely to have the three-dimensional structure required for myosin VI activity.

The researchers next investigated the role of the medial tail and distal tail (MT-DT) regions in step-size generation. Two possible structures for this region have been proposed. The first is that the region forms a dimer with its counterpart on another myosin molecule. The second is that this region is maintained as a single α -helix. In order to test these possibilities, investigators used SAXS to assess the structure of the MT-DT region and found the MT to be a single extended α -helix with sufficient length to account for the large myosin step (Fig. 1). Further experiments with the purified MT-DT in solution showed that it was unlikely to form a dimer under a variety of conditions. Instead, the authors propose that the cargo binding domain is responsible for

bringing two myosin VI molecules together as they work in pairs to carry their cargo. — *Sandy Field*

See: Benjamin J. Spink, Sivaramakrishnan, Jan Lipfert[‡], Sebastian Doniach, and James A Spudich*, "Long single α -helical tail domains bridge the gap between structure and function of myosin VI," *Nat. Struct. Mol. Biol.* **15**(6), 591 (June 2008). DOI: 10.1038/nsmb.1429

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