LETTER

Myosin VI lever arm rotation: Fixed or variable?

Reifenberger et al. (1) reported in PNAS that the myosin VI lever arms tilt by 180° on each step. This contrasts with Sun et al. (2), wherein we found variable degrees of axial and azimuthal tilting, consistent with the known variable step size of myosin VI. There are several differences between the two papers, which are listed here and clarified in a web publication (3).

Reifenberger et al. (1) wrongly explain the difference between the two studies by a choice of analysis hemisphere. Because of the 2-fold symmetry of optical absorption and emission dipoles, any single measurement of polarized fluorescence is consistent with two angles, one in each half of a spherical coordinate system. Reifenberger et al. (1) stated that the dividing plane for quantifying the probe angle must be horizontal. That this statement is incorrect is shown in our original paper on DOPI (ref. 4, figure S8), in figure 3 of Reifenberger et al. (1) (red and orange arrowheads), and by molecules that artifactually jumped between the upper and lower hemispheres described in the PhD thesis of Reifenberger (5) but omitted in Reifenberger et al. (1).

The substantive difference between the two papers is that we found variable angles, whereas Reifenberger et al. (1) found only 180° rotations. Because of the helical disposition of actin binding sites, the interhead angle (and thus the probe angle) depends on the span between the two heads. However, myosin VI has been shown to have a broad distribution of step sizes (refs. 1 and 2; further refs. in ref. 3). To reconcile a broad step size distribution and their constant probe angle, Reifenberger et al. postulated that each myosin VI walks straight with a constant step size, but the step size varies among myosin VI molecules. This argument is fallacious because (*i*) all individual recordings presented in papers from the authors' laboratory (ref. 1, further refs. in ref. 3) show variable steps; (*ii*) the helical structure of actin implies that myosin VI molecules with step size other than 36 nm would walk helically and not straight (refs. in ref. 3); and (*iii*) binding sites for myosin VI to a filament on the glass allow a broad distribution of azimuths (α) [figure 3 in Sun et al. (2)], but the α distributions in Reifenberger et al. (ref. 1, figure 2) show narrow peaks. This result implies severe restriction of myosin VI's landing positions or else strongly biased selection of recordings. Negating all of the previous variable step size data for individual myosin VIs on the basis of the angular measurements of Reifenberger et al. (1) is untenable.

Despite the differences in results, the conclusions of the two studies about myosin VI have some similarities. Reifenberger et al. (1) concluded that the lever and converter are completely uncoupled in the leading head, implying gating of ATP binding by linear strain without torque. We suggested a pliant region between the lever and converter (i.e., not complete uncoupling), similar to that found by crystallography of scallop myosin (referenced in ref. 3). Torque prevents complete rotation of the leading converter and limits ATP binding until the trailing head detaches. The variable lever arm angle we observed is set by the geometrical relationship of the two bound heads and partitioning of the total compliance between this and other flexible regions.

Further issues that distinguish the two studies are presented in our web publication (3).

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