LETTER

Reply to Sun et al.: Myosin VI movement: Wiggly or straight?

In their letter to PNAS (1) and with further material referenced online (2), Sun et al. list three criticisms of our recent work (3). First, they mention that the artifactual dipole transitions between upper and lower hemispheres are reported in J. G. Reifenberger's PhD thesis (4) but not in our paper (3). These events are rare and not associated with myosin VI steps. They falsely appear when the dipole is nearly parallel to the specimen plane due to its intrinsic dipolar degeneracy. We did not include this technical issue in our paper because this phenomenon is sufficiently described by us elsewhere (4, 5). Regardless of the hemisphere selected for data analysis, any polarization methods are prone to similar artifacts. Indeed, similar flipping events were observed in all three traces of Sun et al.'s original article (Figure 2, bottom panel, open circles, in ref. 6). Sun et al. also state that there might be a bias for selection of recordings in our paper. In fact, there is no data elimination or biased selection of the recordings in our work. Our only criterion was whether there was myosin VI motility or not.

The second criticism concerns the hemisphere chosen for our analysis. We do not claim that there is a correct or an incorrect analysis hemisphere if the analysis is done carefully. Any hemisphere choice will impose problems due to dipolar degeneracy especially if there is no additional information (e.g., displacement) (5). Our data for the cys^{66}-cys^{73} showed different values than what is reported in Sun et al.'s paper (6). We merely showed in our paper that the differences could be accounted by the choice of hemispheres and that upon converting our data to their hemisphere we got similar trends in both α and β (3, 6). This can further be verified by reanalyzing sample traces of Sun et al. (6) using our geometric definitions (3, 5).

Finally, the third criticism deals with the mechanics of myosin VI's motion along actin. Sun et al. are concerned that we did not observe any azimuthal rotations despite the variable step sizes of myosin VI. Yet there is no evidence in the literature to assume that the suggested wobbly movements of myosin VI can explain its variable step size. For instance, myosin V does not have such variable step sizes although it has the azimuthal freedom similar to myosin VI. The proposed wobbly movements are not real physical events; they falsely appear due to an erroneous geometric transformation (6).

In summary, we argue that the uncoupling and the 180° swinging of the lever arm could possibly account for the large variable step sizes. Furthermore we do not rule out that our long integration time could have missed intermediate conformations picked up by Sun et al. (6). In our model, the lever arm is uncoupled, but still tethered, and therefore cannot rapidly couple into the conformation necessary for ATP binding until the rear head releases from actin. This uncoupling, combined with the large swinging of the lever arm, accounts for the large and variable step size of myosin VI.

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The authors declare no conflict of interest.

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