What is Myosin VI and Why is it in my Ears?

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- Title: Robust Mechanosensing and Tension Generation by Myosin VI
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The one takeaway most people get from their high school biology class is that the mitochondria is the powerhouse of the cell. If mitochondria is the powerhouse, then myosin VI is the postal service and the highway administration of the cell. Myosin VI is a micrometer-scale motor that's responsible for intra-cellular transportation and maintaining/manipulation cytoplasmic structure. Myosin VI functions like an engine by hydrolyzing (think burning) ATP (fuel), then releasing ADP (think emissions from your car's tailpipe). It is understood that Myosin VI is capable of making forward steps to traffic and is capable of becoming an anchor. It is proposed that the anchor function of myosin VI is crucial in maintaining the intra-cellular organelle like Stereocillia [1]. See, Stereocilla are very, very small protrusions on the sensory cells in your ears. These protrusions have to exist under an internal tension for them to be able to detect the vibrations, which allows you to hear [2]. The myosin VI motors in your cells are understood to be responsible for generating and maintaining this tension. This paper seeks to create a new model for how myosin VI acts *in vitro* (lone standing in glass).

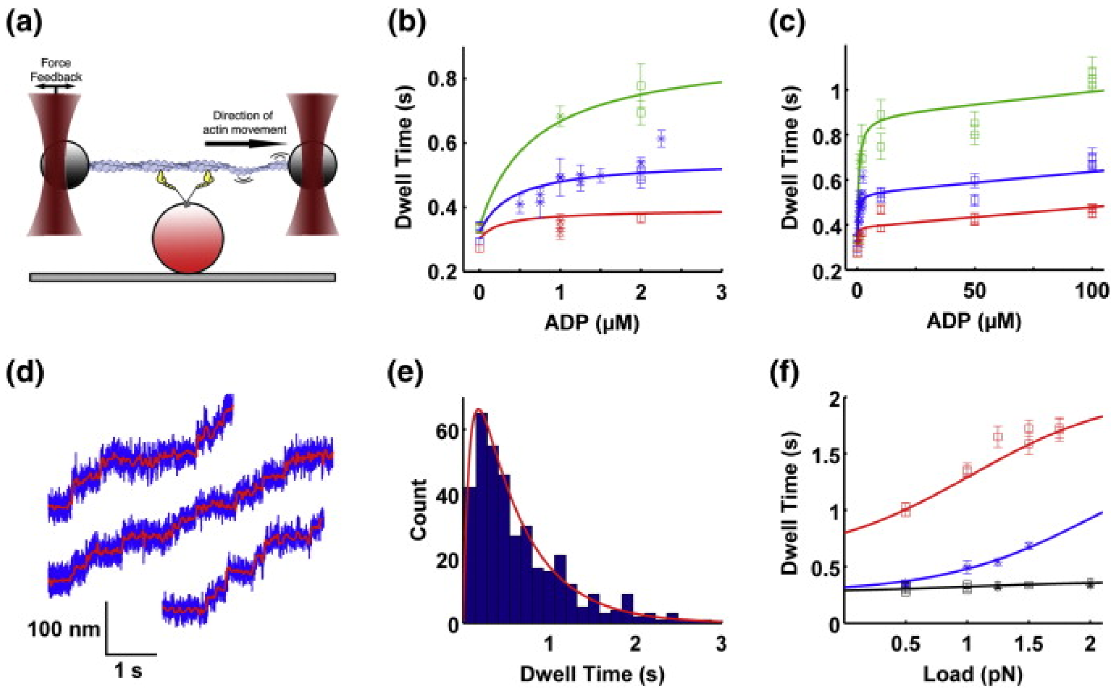
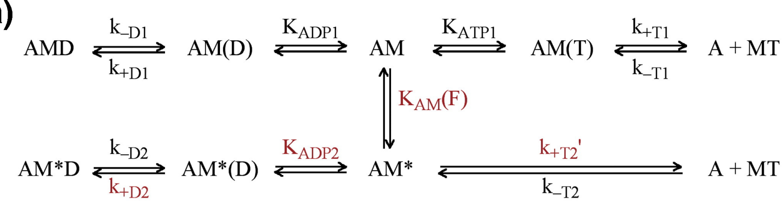


FIG. 1. a) Shows the experiment set-up with the two optical traps holding the beads, the actin filament connecting the beads, the surface bound bead with the myosin bound to the bead. b) Shows the dwell time (time between steps) vs the concentration of ADP in the flow cell in the small ranges of ADP. The ATP concentration is set to $2mM$. The green, blue, and red data points are for loads of $1.5pN$, $1.0pN$, and $0.5pN$, respectively. c) is the same as b) but with the complete range for ADP variations. d) Shows a sample of the raw data collected and shows what is determined as a step. The conditions for this data was concentrations of $1.5mM$ of ATP and $1\mu M$ of ADP, a load of $1pN$. e) Shows a histogram for dwell time of the motors from the data from d). f) Shows the dwell time's dependence on the load of the motor. The black line represents $2nM$ ATP, the blue line represents concentrations of $1.5mM$ ATP + $1\mu M$ ADP, and the red line represents a pure concentration of $0.1mM$ ATP.

To further understand and accurately model myosin VI's dual functionality, the researchers used two independent optical traps to hold two polystyrene beads connected by a actin filament. A optical trap is essentially, a micrometer scale tractor beam from Star Wars. If you haven't seen Star Wars (or don't remember), the tractor beam / optical trap (OT) can trap an object in the focal point of a laser. Once trapped, the trapped object can be moved around by changing the focal point of the laser. Coming back to the experiment, after trapping the two beads, they would lower the filament onto a myosin VI bound to a platform bound bead. Then, as seen in figure 1 (a), they would apply a constant force on one side of the actin to make the myosin walk along it. In doing so they could see and quantify how much the myosin would pull against the actin. In figure 1 (b and c), you can see that the researchers found that the myosin's movement would substantially slow in the presence of more ADP (burnt fuel), but this effect would drop off after reaching a 3μM concentration. In (d) you can see that the heavier the load for the motor, the slower it would move. The researchers also noted that the hyperbolic curves shown in the data imply that myosin VI has a multi-step process for binding with ADP.

Using their data, the researchers built a new kinetic model for myosin VI, seen in figure 2. While we wont be mucking with the specifics of this model, we can go into the basic ideas in it and the behavior predicted by it. The model the researchers created functions with the idea that the myosin will be either in the M state or the M^* state. The balance for which state the myosin is in is dependent on the force being exerted by the myosin. So when the myosin is pulling a very heavy load, it is more likely to be converted to the M^* state. This implies that the M^* state has a far greater affinity for binding with ADP and, inversely, the M state prefers to bond with ATP. This can be roughly thought of as the M^* being the anchor state and the M state being the walking state of the motor.

(a)



(b)

	$k_{-D1} = k_{-D2}$ (s^{-1})	k_{+D1} (s^{-1})	K_{ADP1} (μM)	K_{ATP1} (μM)	k_{+T1} (s^{-1})	K_{ADP2} (μM)	k_{+D2} (s^{-1})	k_{+T2}' (μM^{-1} s^{-1})	$K_{AM(0)}$	D (nm)
Value	4.0	366	2200	5600	176	0.18	3.6	$5.6\text{x}10^{-3}$	1.0	-6.7
Error						0.03	0.8	$0.3\text{x}10^{-3}$	0.1	0.2

FIG. 2. The kinetic model made for myosin VI under load. a) is the kinetic scheme made by the researchers. M represents the myosin motor, A represents actin, D represents ADP, and T represents ATP. Any letter adjacent to M or M* shows that the respective of "foot" of the myosin. So, AMD represents myosin's back foot connected to actin, myosin is in its lesser load state (no star), and the forward foot of the myosin is bound to ADP. b) Previous research gives the values in black then the calculated values are shown in red..

The key takeaways from this paper are that myosin, when it is a typical cell environment, (1) at low loads make efficient steps along actin that have dwell times that depends of the force on the load. This dependence (2) results in increasing dwell times until ∼2 pN. Going above ∼2.5 pN, (3) myosin VI turns into a dynamic anchor that dampens forces by making intermittent back steps. This sweet spot at a force of $2 - 2.5$ pN shows that myosin can hold and generate tension *in vivo*. This dual functionality represented by the motor's stepping and anchoring behavior strongly implies that myosin VI is crucial in maintaining the structural integrity of cells that have to be held in tension and how it is able to play such a crucial role in intra-cellular transportation.

[1] A. R. D. Peiying Chuan, James A. Spudich, Robust mechanosensing and tension generation by myosin vi, Journal of Molecular Biology **405**, Pages 105 (7 January 2011).

[2] F. Buss and J. Kendrick-Jones, How are the cellular functions of myosin vi regulated within the cell?, Biochemical and Biophysical Research Communications **369**, 165 (2008), ebashi Memorial Issue.