Supplementary Material for: Load and P_i Control Flux Through the Branched Kinetic Cycle of Myosin V Neil M. Kad, Kathleen M. Trybus and David M. Warshaw

Figure 1S



Backstep size distributions in the presence of P_i show similar properties to those in the absence of P_i .

(a) The backstep size distribution in the presence of P_i is broadly distributed. Although the distribution can be fit by a single Gaussian, we elected to fit the distribution to a double Gaussian based on the observations made in the absence of Pi with one population fixed at -36nm. The fit in (a) is therefore described by one Gaussian at -36(fixed)±8(S.D.)nm, amplitude=15 and the second at -20±12(S.D.)nm, amplitude=40. These backsteps are preceded by two populations of forward steps (b), one corresponding to a normal 36(fixed)±7(S.D.)nm, amplitude=46 and the second corresponding to a foreshortened step of 23±4(S.D.)nm, amplitude=18. Pairwise analysis of these data is shown in panel (c) where the preceding forward step is subtracted from the backstep. The result of this analysis is a single Gaussian centered on 5±11(S.D.)nm, amplitude=35. This suggests that backsteps are almost complete reversals of the preceding forward step. Interestingly, the 5nm offset from zero indicates that upon reversal, the detached head misses its former binding site by approximately 1 actin monomer on average. Following a backstep, the next forward step (d) is again characterized by two distributions centered on 23±4(S.D.)nm, amplitude=17 and 38±7(S.D.)nm, amplitude=49, similar to the distribution seen in (b). As in the absence of P_i (see Discussion), the population of shorter forward steps (i.e. 23 nm) may occur as a result of a partial backstep forcing the lead head to bind azimuthally relative to the trailing head, so that upon its next forward step, the trailing head binds to actin in register with the lead head, displacing the motor's center of mass less than 36 nm (see Fig. 8 right side).