

# Supporting Information

Hinczewski et al. 10.1073/pnas.1312393110

## SI Text

In *SI Text*, we provide the details of the theory that nearly quantitatively explains the complex kinetic pathways in the stepping dynamics of myosin V (MyoV). Because this SI Text is long, containing technical details of the calculations, we begin with a collection of the most important equations, which were used to make the predictions described in the main text. The subsequent sections describe the details leading to these equations.

### 1. Summary of Key Equations for MyoV Dynamics

#### First Passage and Binding.

$$t_{\text{fp}}^{\pm} = \frac{1}{4\pi a D_{\text{h}} \mathcal{P}(\mathbf{r}_{\pm})}, \quad t_{\text{Tb}} = t_{\text{h}} + \frac{t_{\text{fp}}^{+}}{1+b\alpha}, \quad t_{\text{Lb}} = \frac{t_{\text{fp}}^{+}}{b+\alpha}, \quad \alpha = t_{\text{fp}}^{+}/t_{\text{fp}}^{-} \quad [\text{S9, S14, and S19}]$$

#### Kinetic Pathway Probabilities.

$$\mathcal{P}_{\text{f}} = \frac{g}{1+g} \frac{t_{\text{d1}}^2}{(1+b\alpha)(t_{\text{d1}}+t_{\text{h}})(t_{\text{d1}}+t_{\text{Tb}}-t_{\text{h}})}, \quad \mathcal{P}_{\text{Tb}} = b\alpha\mathcal{P}_{\text{f}} \quad [\text{S15 and S16}]$$

$$\mathcal{P}_{\text{Ls}} = \frac{1}{1+g} \frac{bt_{\text{d1}}}{(b+\alpha)(t_{\text{d1}}+t_{\text{Lb}})}, \quad \mathcal{P}_{\text{b}} = b^{-1}\alpha\mathcal{P}_{\text{Ls}} \quad [\text{S20 and S21}]$$

#### Average Step Shape.

$$\mathcal{P}_{\text{Tb}}^{+}(t) = \frac{t_{\text{h}}(1-e^{-t/t_{\text{h}}}) - t_{\text{fp}}^{+}(1+b\alpha)^{-1}(1-e^{-(1+b\alpha)t/t_{\text{fp}}^{+}})}{t_{\text{h}}(1+b\alpha) - t_{\text{fp}}^{+}}, \quad \mathcal{P}_{\text{Tb}}^{-}(t) = b\alpha\mathcal{P}_{\text{Tb}}^{+}(t) \quad [\text{S22 and S23}]$$

$$\langle \delta z(t) \rangle = (\mu_z + \Delta)(1 - \mathcal{P}_{\text{Tb}}^{+}(t) - \mathcal{P}_{\text{Tb}}^{-}(t))(1 - e^{-t/t_{\text{r}}}) + 2\Delta\mathcal{P}_{\text{Tb}}^{+}(t) \quad [\text{S24}]$$

$$\mu_z = l_{\text{p}} \left(1 - e^{-L/l_{\text{p}}}\right) (\coth \nu_c - \nu_c^{-1}) \cos \theta_c \quad [\text{S25}]$$

#### Mean Run Length and Velocity.

$$z_{\text{run}} = \nu_{\text{run}} t_{\text{run}}, \quad \nu_{\text{run}} \approx \frac{\Delta}{t_{\text{d1}}} \left( \frac{1}{1+b\alpha} - \frac{\alpha}{g(b+\alpha)} \right), \quad t_{\text{run}} \approx \frac{gt_{\text{d1}}^2}{t_{\text{Lb}} + gt_{\text{Tb}}} \quad [\text{S26–S28}]$$

#### Equilibrium End-Point Probability Distribution.

$$T \approx 1 + \frac{20\nu_c}{20+7\kappa\nu_c}, \quad T' = \sqrt{(T'_x)^2 + (T'_z)^2} \quad [\text{S41 and S44}]$$

$$T'_x = T \sin \theta_c + \beta F L \sin \theta_F, \quad T'_z = T \cos \theta_c - \beta F L \cos \theta_F \quad [\text{S44}]$$

$$\mathcal{P}(\mathbf{r}_{\pm}) \approx \frac{(3\kappa(7\kappa+20)+200)T'}{1,600\pi L^2 \Delta \sinh T'} I_0 \left( T'_x \sqrt{1 - \frac{\Delta^2}{4L^2}} \right) e^{\pm \frac{T'_z \Delta}{2L}} \quad [\text{S55}]$$

#### Stall Force.

$$F_{\text{stall}} = \frac{k_B T}{\cos \theta_F} \left( \frac{T}{L} \cos \theta_c + \frac{1}{\Delta} \log \frac{g-1 + \sqrt{(g-1)^2 + 4gb^2}}{2b} \right) \quad [\text{S59}]$$

### 2. First Passage Times, Binding Probabilities, and Experimental Observables

**Mean First Passage Time to a Target Site.** After the detachment of one of the MyoV heads from the polar actin tracks, there are two potential actin target sites where the head could rebind, at positions  $\mathbf{r}_{\pm} = \pm \Delta \hat{\mathbf{z}}$  (Fig. 1B). The axis  $\hat{\mathbf{z}}$  is oriented from the minus to plus end of the actin filament, so we denote  $\mathbf{r}_{+}$  and  $\mathbf{r}_{-}$  as the forward and backward target sites, respectively. Before dealing with the full complexity of the diffusive search and binding for multiple targets (with binding probabilities dependent on the head chemical state), we solve a simpler problem: What is the mean first passage time for the free end of MyoV to reach a sphere of radius  $a$  around one of the target sites, for example,  $\mathbf{r}_{+}$ ? (The derivation below will hold analogously for  $\mathbf{r}_{-}$ , with the  $+$  superscripts and subscripts replaced by  $-$ .)

Let  $f_{\text{fp}}(\mathbf{r}, \mathbf{r}'; t)$  be the distribution of first passage times for the free end to go from an initial position  $\mathbf{r}$  to some final position  $\mathbf{r}'$ . Using the renewal approach (1), the first passage time distribution can be related to the Green's function  $G(\mathbf{r}, \mathbf{r}'; t)$  describing the probability of diffusing from  $\mathbf{r}$  to  $\mathbf{r}'$  in time  $t$ . Choose a final position on a sphere of radius  $a$  around the target site  $\mathbf{r}_{+}$ , so that  $\mathbf{r}' = \mathbf{r}_{+} + a\hat{\mathbf{e}}$ , where  $\hat{\mathbf{e}}$  is any unit vector. The renewal approach relates  $f_{\text{fp}}$  and  $G$  through the integral equation

$$G(\mathbf{r}, \mathbf{r}_{+} + a\hat{\mathbf{e}}; t) = \int_0^t dt' \int a^2 d\hat{\mathbf{e}}' f_{\text{fp}}(\mathbf{r}, \mathbf{r}_{+} + a\hat{\mathbf{e}}'; t') \times G(\mathbf{r}_{+} + a\hat{\mathbf{e}}', \mathbf{r}_{+} + a\hat{\mathbf{e}}; t-t'). \quad [\text{S1}]$$

The physical meaning of the equation above is that the Green's function for going from  $\mathbf{r}$  to a particular point  $\mathbf{r}_{+} + a\hat{\mathbf{e}}$  on the target sphere consists of paths that make first passage at some point  $\mathbf{r}_{+} + a\hat{\mathbf{e}}'$  on the target sphere at time  $t' \leq t$  and then diffuse from  $\mathbf{r}_{+} + a\hat{\mathbf{e}}'$  to  $\mathbf{r}_{+} + a\hat{\mathbf{e}}$  in time  $t-t'$ . Because Eq. S1 is difficult to solve analytically, we make three simplifications, motivated by the observation that the capture radius  $a$  is small compared with all other length scales in the problem: (i) we approximately average over all final positions on the target sphere, replacing  $\mathbf{r}_{+} + a\hat{\mathbf{e}}$  with  $\mathbf{r}_{+}$  on both sides of Eq. S1; (ii) we assume  $f(\mathbf{r}, \mathbf{r}_{+} + a\hat{\mathbf{e}}'; t')$  does not vary appreciably with  $\hat{\mathbf{e}}'$  so that it can be replaced by  $f_{\text{fp}}^{+}(\mathbf{r}; t')/4\pi a^2$ , where  $f_{\text{fp}}^{+}(\mathbf{r}; t')$  is the first passage time distribution for reaching any point on a target sphere of radius  $a$  around  $\mathbf{r}_{+}$ , starting from  $\mathbf{r}$ ; and (iii) the Green's function on the right-hand side of Eq. S1 will not depend significantly on the specific unit vector  $\hat{\mathbf{e}}'$  defining the starting position, so we replace  $\hat{\mathbf{e}}'$  in the argument of the Green's function by a fixed unit vector  $\hat{\mathbf{z}}$ . With these approximations, Eq. S1 becomes

$$G(\mathbf{r}, \mathbf{r}_{+}; t) \approx \int_0^t dt' f_{\text{fp}}^{+}(\mathbf{r}; t') G(\mathbf{r}_{+} + a\hat{\mathbf{z}}, \mathbf{r}_{+}; t-t'). \quad [\text{S2}]$$

The above renewal equation can be solved by Laplace-transforming both sides to yield

$$\tilde{f}_{\text{fp}}^+(\mathbf{r}; s) \approx \frac{\tilde{G}(\mathbf{r}, \mathbf{r}_+; s)}{\tilde{G}(\mathbf{r}_+ + a\hat{\mathbf{z}}, \mathbf{r}_+; s)}, \quad [\text{S3}]$$

where  $\tilde{f}_{\text{fp}}^+$  and  $\tilde{G}$  are Laplace-transformed functions. For example,  $\tilde{G}(\mathbf{r}, \mathbf{r}'; s) = \int_0^\infty dt e^{-st} G(\mathbf{r}, \mathbf{r}'; t)$ , and a similar equation holds for  $\tilde{f}_{\text{fp}}^+$ . The derivative of  $\tilde{f}$  with respect to  $s$  at  $s=0$  is related to the mean first-passage time  $t_{\text{fp}}^+(\mathbf{r})$  to arrive at the target sphere of radius  $a$  around  $\mathbf{r}_+$ :

$$-\frac{\partial}{\partial s} \tilde{f}_{\text{fp}}^+(\mathbf{r}; s) \Big|_{s=0} = \int_0^\infty dt t f_{\text{fp}}^+(\mathbf{r}; t) = t_{\text{fp}}^+(\mathbf{r}). \quad [\text{S4}]$$

We can simplify Eq. S3 by taking advantage of time scale separation in the system. For  $t \gg t_r$ , the relaxation time of the two-legged polymer, the Green's function for going from an initial to a final position approaches the equilibrium probability distribution of finding the free end at the final position,  $G(\mathbf{r}, \mathbf{r}'; t) \rightarrow \mathcal{P}(\mathbf{r}')$  as  $t \rightarrow \infty$ . In Laplace space, this implies that the Green's function can be decomposed into two contributions:

$$\begin{aligned} \tilde{G}(\mathbf{r}, \mathbf{r}'; s) &\approx \int_0^{t_r} dt e^{-st} G(\mathbf{r}, \mathbf{r}'; t) + (s^{-1} - t_r) \mathcal{P}(\mathbf{r}') \\ &\equiv \tilde{G}_0(\mathbf{r}, \mathbf{r}'; s) + (s^{-1} - t_r) \mathcal{P}(\mathbf{r}'). \end{aligned} \quad [\text{S5}]$$

For  $\tilde{G}(\mathbf{r}, \mathbf{r}_+; s)$  in the numerator of Eq. S3, we assume the initial  $\mathbf{r}$  is not in the immediate vicinity of the target  $\mathbf{r}_+$  (which is generally the case for a MyoV diffusive search), so the time to reach the target will be much larger than the relaxation time  $t_r$ . Hence,  $\tilde{G}_0(\mathbf{r}, \mathbf{r}_+; s)$  will be negligible, because  $G(\mathbf{r}, \mathbf{r}_+; t)$  is near zero on the time scale  $t < t_r$ . Thus, we can approximate the numerator of Eq. S3 as

$$\tilde{G}(\mathbf{r}, \mathbf{r}_+; s) \approx (s^{-1} - t_r) \mathcal{P}(\mathbf{r}_+). \quad [\text{S6}]$$

For the denominator of Eq. S3,  $\tilde{G}(\mathbf{r}_+ + a\hat{\mathbf{z}}, \mathbf{r}_+; s)$ , the situation is more complicated, because the initial and final positions are separated by a small distance  $a$ ; hence, there will be contributions to  $\tilde{G}_0$  at short times. In the limit  $a \rightarrow 0$ , the paths between  $\mathbf{r}_+ + a\hat{\mathbf{z}}$  and  $\mathbf{r}_+$  involve only a fast microscopic rearrangement of the free end, without significant configurational changes in the rest of the structure. If we model the free end as a particle with diffusion constant  $D$ , the Green's function in the short time limit can be approximated as (1)

$$G(\mathbf{r}_+ + a\hat{\mathbf{z}}, \mathbf{r}_+; t) \approx (4\pi Dt)^{-3/2} \exp(-a^2/(4Dt)). \quad [\text{S7}]$$

Substituting Eq. S7 into the integral for  $\tilde{G}_0$ , we get an expression for the denominator:

$$\begin{aligned} \tilde{G}(\mathbf{r}_+ + a\hat{\mathbf{z}}, \mathbf{r}_+; s) &\approx \frac{t_a}{4\pi a^3} \operatorname{erfc}\left(\frac{1}{2} \sqrt{\frac{t_a}{t_r}}\right) + (s^{-1} - t_r) \mathcal{P}(\mathbf{r}_+) \\ &\approx \frac{t_a}{4\pi a^3} + (s^{-1} - t_r) \mathcal{P}(\mathbf{r}_+), \end{aligned} \quad [\text{S8}]$$

where  $t_a = a^2/D$  is a microscopic time scale describing how long it takes a particle of diffusivity  $D$  to move a distance  $a$ . The second approximation in Eq. S8 assumes  $t_a \ll t_r$ , which is justified by a simple calculation: Let us set  $D = D_h$ , where  $D_h = 5.7 \times 10^{-7} \text{cm}^2/\text{s}$  is the diffusion constant of the MyoV head, as derived from the Protein Data Bank structure 1W8J (2) using the program

HYDROPRO (3). For  $a = 1$  nm, the resulting microscopic time scale is  $t_a = 18$  ns, which is significantly smaller than the relaxation time  $t_r \sim \mathcal{O}(1 \mu\text{s})$  of the entire structure (estimates of  $t_r$  are provided in the next subsection).

Using Eqs. S6 and S8 in Eq. S3, and then evaluating the derivative in Eq. S4, we obtain the final approximate expression for the mean first passage time:

$$t_{\text{fp}}^+ = \frac{1}{4\pi a D_h \mathcal{P}(\mathbf{r}_+)}. \quad [\text{S9}]$$

We have dropped the  $\mathbf{r}$  dependence in the notation for  $t_{\text{fp}}^+(\mathbf{r})$ , because the first passage time result is independent of the initial position  $\mathbf{r}$ . This reflects the underlying assumption that the configurational relaxation time  $t_r \ll t_{\text{fp}}^+$ , so the free end loses memory of its initial position during the long diffusive search. An analogous result holds for the mean first passage time  $t_{\text{fp}}^-$  to the backward target site, with  $\mathbf{r}_+$  replaced by  $\mathbf{r}_-$  in Eq. S9. A result similar in spirit to Eq. S9 but without the benefit of derivation was conjectured earlier (4).

To validate the approximation underlying Eq. S7, we performed Brownian dynamics simulations on a bead-spring semiflexible polymer model of two-legged MyoV (further details are provided in *SI Text, Relaxation Times*). By generating many individual trajectories of the detached polymer end point diffusing a small distance  $a$  from  $\mathbf{r}_+$  to some point  $\mathbf{r}_+ + a\hat{\mathbf{e}}$ , we numerically reconstruct the corresponding Green's function (Fig. S1). The excellent fit of the assumed form in Eq. S7 for several values of  $a$  to the numerical results justifies the approximation.

**Relaxation Times.** To estimate the relaxation time  $t_r$  of the two-legged MyoV structure, we performed Brownian dynamics (5) simulations of a bead-spring semiflexible polymer model. Each leg consists of 17 beads of diameter  $d = 2$  nm, with an additional bead at the flexible joint between the legs. The beads are connected through harmonic springs of stiffness  $200 k_B T/\text{nm}^2$ , where  $k_B$  is Boltzmann's constant, and  $T$  is temperature. Each leg has a bending elasticity described by a persistence length  $l_p = 50 - 400$  nm. Initially, the end beads are fixed at the two binding sites. The end tangent of the leading leg (the unit vector oriented between the centers of the first two beads) is subject to a harmonic constraint of strength  $k_B T \nu_c$  along  $\hat{\mathbf{u}}_c$  (at an angle of  $\theta_c = 60^\circ$  from the actin filament), with  $\nu_c = 50 - 180$ . The joint between the legs is subject to a backward load force of  $F$ . The beads are coupled hydrodynamically through the Rotne-Prager tensor (6), and their positions evolve in time numerically according to the Langevin equation. Each simulation lasts  $12 \mu\text{s}$ , where both end beads are bound during the first  $2.4 \mu\text{s}$  and the trailing leg end bead is allowed to diffuse freely during the remaining time. By averaging a large number of individual simulations (1,000–1,250 runs for each distinct parameter set of  $l_p$  and  $\nu_c$ ), we can extract the mean relaxation time  $t_r$  for the  $z$ -axis position of the trailing leg end bead to reach equilibrium after detachment.

Fig. S2 shows the resulting values of  $t_r$  for  $\nu_c = 50$  and  $\nu_c = 180$ , with  $A$  plotting  $t_r$  as a function of  $l_p$  and  $B$  plotting  $t_r$  as a function of backward load force  $F$  at  $l_p = 310$  nm. In the absence of load,  $t_r \approx 5 \mu\text{s}$  for both values of  $\nu_c$  over the entire plotted range of  $l_p$  (corresponding to the semiflexible regime  $l_p > L$ ). Because relaxation of MyoV requires a rotational reorientation of a stiff, two-legged structure (with each leg of contour length  $L = 35$  nm), we expect that  $t_r$  should fall in the range between the rotational diffusion time  $t_{\text{rod}}(L)$  of a rigid rod of contour length  $L$  and  $t_{\text{rod}}(2L)$ , the time for a rigid rod of length  $2L$ . Analytically,  $t_{\text{rod}}(L)$  can be approximated as follows (7):

$$t_{\text{rod}}(L) = \frac{\pi \eta L^3}{3 \ln(L/2d)}, \quad [\text{S10}]$$

where  $\eta$  is the viscosity of water. The resulting rotational diffusion times  $t_{\text{rod}}(L) \approx 2.2 \mu\text{s}$  and  $t_{\text{rod}}(2L) \approx 13.3 \mu\text{s}$  are marked as black dashed lines in Fig. S2A, which establishes that  $t_{\text{rod}}(L) < t_r < t_{\text{rod}}(2L)$ . A more precise analytical comparison can be made with the rotational diffusion time  $t_w$  of a structure consisting of two rigid rods of  $L$  connected by a flexible hinge, which has been estimated by Wegener (8):

$$t_w(L) \approx 1.79 \frac{\pi\eta L^3}{\ln(2L/d)}. \quad [\text{S11}]$$

The resulting value,  $t_w(L) = 4.6 \mu\text{s}$ , which is marked as a red dashed line in Fig. S2A, is in good agreement with the simulation results. With a load force  $F$  applied to MyoV, the equilibrium position of the end point after detachment is shifted closer to the initial binding site. As a result, the relaxation times become shorter, as seen in Fig. S2B. In all cases,  $t_r$  is at least two orders of magnitude smaller than the typical first passage times to the binding site, which is consistent with the approximation used to derive Eq. S9.

**Binding Probabilities.** When MyoV is in the waiting state, with both heads bound to ADP and strongly associated with actin, we can have one of two scenarios for initiating a diffusive search: (I) ADP is released from the trailing head (TH) and quickly replaced by ATP, leading to the dissociation of the TH from actin, in which this detachment through ADP release/ATP binding has an overall rate of  $t_{\text{d1}}^{-1}$ , and (II) less frequently, the leading head (LH) detaches without ADP release, which occurs at a rate of  $t_{\text{d2}}^{-1} \ll t_{\text{d1}}^{-1}$ . The gating parameter  $g = t_{\text{d2}}/t_{\text{d1}} \gg 1$  describes the probabilities of the two scenarios occurring, which are  $g(1+g)^{-1}$  for I and  $(1+g)^{-1}$  for II.

Let us consider scenario I, which can lead either to a forward step if the TH rebinds to  $\mathbf{r}_+$  or to a trailing foot stomp if the TH binds to  $\mathbf{r}_-$ . Denote the probabilities of these two binding events as  $\mathcal{P}_f$  and  $\mathcal{P}_{\text{Ts}}$ . For the TH to bind to actin, three conditions must be fulfilled:

- i) The TH must hydrolyze ATP, which occurs at a hydrolysis rate of  $t_h^{-1}$ .
- ii) Subsequently, the TH must reach the capture radius  $a$  of one of the binding sites. For  $\mathbf{r}_+$ , it reaches the capture radius with a rate of  $(t_{\text{fb}}^+)^{-1}$  and then binds. For  $\mathbf{r}_-$ , it reaches the capture radius with a rate of  $(t_{\text{fb}}^-)^{-1}$ , but binding will only occur with probability  $b$ , reflecting the penalty for wrong head orientation after the recovery stroke. Thus, the effective rate of capture at the backward site is  $b(t_{\text{fb}}^-)^{-1}$  with  $b \ll 1$  (Table 1).
- iii) During the entire diffusive search, the LH must not detach from actin or the entire MyoV structure will dissociate from the filament and the run is terminated. The detachment rate, assumed to be ATP-independent, is given by  $t_{\text{d1}}^{-1}$ .

Requirements *i* and *ii* by themselves, and the assumption that individual events are Poisson-distributed, lead to probability distributions  $f_{\text{Tb}}^\pm(t)$  for the TH binding time to the  $\mathbf{r}_\pm$  target sites:

$$f_{\text{Tb}}^+(t) = \int_0^t dt' t_h^{-1} e^{-t'/t_h} (t_{\text{fb}}^+)^{-1} e^{-(t-t')} \left[ (t_{\text{fb}}^+)^{-1} + b(t_{\text{fb}}^-)^{-1} \right] \\ = \frac{e^{-t/t_h} - e^{-t(1+b\alpha)/t_{\text{fb}}^+}}{t_h(1+b\alpha) - t_{\text{fb}}^+}, \quad [\text{S12}]$$

$$f_{\text{Tb}}^-(t) = \int_0^t dt' t_h^{-1} e^{-t'/t_h} b(t_{\text{fb}}^-)^{-1} e^{-(t-t')} \left[ (t_{\text{fb}}^+)^{-1} + b(t_{\text{fb}}^-)^{-1} \right] \\ = b\alpha f_{\text{Tb}}^+(t), \quad [\text{S13}]$$

where  $\alpha = t_{\text{fb}}^+/t_{\text{fb}}^-$ . The integrals in Eq. S12 are convolutions of the probability that hydrolysis occurs at some time  $t'$  and the probability of subsequent capture at a target site after a time interval  $t-t'$ . The average time to bind,  $t_{\text{Tb}}$ , is the same for both sites:

$$t_{\text{Tb}} = \frac{\int_0^\infty dt' t' f_{\text{Tb}}^+(t')}{\int_0^\infty dt' f_{\text{Tb}}^+(t')} = \frac{\int_0^\infty dt' t' f_{\text{Tb}}^-(t')}{\int_0^\infty dt' f_{\text{Tb}}^-(t')} = t_h + \frac{t_{\text{fb}}^+}{1+b\alpha}. \quad [\text{S14}]$$

Using Eq. S12, it is straightforward to incorporate requirement *iii* and derive the probabilities  $\mathcal{P}_f$  and  $\mathcal{P}_{\text{Ts}}$ :

$$\mathcal{P}_f = \frac{g}{1+g} \int_0^\infty dt e^{-t/t_{\text{d1}}} f_{\text{Tb}}^+(t) = \frac{g}{1+g} \frac{t_{\text{d1}}^2}{(1+b\alpha)(t_{\text{d1}}+t_h)(t_{\text{d1}}+t_{\text{Tb}}-t_h)}. \quad [\text{S15}]$$

$$\mathcal{P}_{\text{Ts}} = \frac{g}{1+g} \int_0^\infty dt e^{-t/t_{\text{d1}}} f_{\text{Tb}}^-(t) = b\alpha \mathcal{P}_f. \quad [\text{S16}]$$

In scenario II, ATP hydrolysis is not required for rebinding, because the detached LH retains ADP and is in a state that can strongly associate with actin. The head orientation is now favorable for binding to the backward site, so the binding penalty  $b$  exists for  $\mathbf{r}_+$  instead of  $\mathbf{r}_-$ . The free LH can bind to  $\mathbf{r}_+$ , a leading foot stomp with probability  $\mathcal{P}_{\text{Ls}}$ , or it can bind to  $\mathbf{r}_-$ , a backward step with probability  $\mathcal{P}_b$ . The LH analogs to Eqs. S12–S16 can be obtained from these equations by the substitutions  $t_h = 0$ ,  $b(t_{\text{fb}}^-)^{-1} \rightarrow (t_{\text{fb}}^-)^{-1}$ , and  $(t_{\text{fb}}^+)^{-1} \rightarrow b(t_{\text{fb}}^+)^{-1}$ . The results are

$$f_{\text{Lb}}^+(t) = b(t_{\text{fb}}^+)^{-1} e^{-t(b+\alpha)/t_{\text{fb}}^+}, \quad [\text{S17}]$$

$$f_{\text{Lb}}^-(t) = b^{-1}\alpha f_{\text{Lb}}^+(t), \quad [\text{S18}]$$

$$t_{\text{Lb}} = \frac{t_{\text{fb}}^+}{b+\alpha}, \quad [\text{S19}]$$

$$\mathcal{P}_{\text{Ls}} = \frac{1}{1+g} \frac{bt_{\text{d1}}}{(b+\alpha)(t_{\text{d1}}+t_{\text{Lb}})}, \quad [\text{S20}]$$

$$\mathcal{P}_b = b^{-1}\alpha \mathcal{P}_{\text{Ls}}. \quad [\text{S21}]$$

The final kinetic pathway, termination by complete dissociation from actin, occurs when the diffusive search in any of the four pathways above cannot be completed before the bound leg detaches. The termination probability is  $\mathcal{P}_t = 1 - \mathcal{P}_f - \mathcal{P}_{\text{Ts}} - \mathcal{P}_{\text{Ls}} - \mathcal{P}_b$ .

From Eqs. S15, S16, S20, and S21, one can derive the pathway probability ratios shown in Eq. 4. The results for the ratios have been simplified under the assumption that  $t_{\text{d1}} \gg t_{\text{Lb}}, t_{\text{Tb}}$ , which is generally valid.

**Average Step Shape.** For comparison with the experiment of Dunn and Spudich (9), we will consider the average step trajectory  $\langle \delta z(t) \rangle$  of the TH along  $\hat{\mathbf{z}}$  after detachment from actin, where  $\delta z(t) \equiv z(t) - z(0)$  and the initial position is the backward binding site,  $z(0) = \hat{\mathbf{z}} \cdot \mathbf{r}_- = -\Delta$ . In the ensemble of all possible trajectories at time  $t$  after detachment (with at least one head bound to actin), there will be two subpopulations: those trajectories where the TH is still unbound and those where the TH has bound either to the backward site  $\mathbf{r}_-$  or to the forward site  $\mathbf{r}_+$ . In this calculation, we ignore the small fraction of trajectories that lead

to complete dissociation of the motor because these are not counted as completed steps, and hence do not contribute to the experimental measurement of  $\langle \delta z(t) \rangle$ . The fraction  $\mathcal{P}_{\text{Tb}}^{\pm}(t)$  of TH trajectories that has bound to  $\mathbf{r}_{\pm}$  by time  $t$  is

$$\mathcal{P}_{\text{Tb}}^{+}(t) = \int_0^t dt' f_{\text{Tb}}^{+}(t') \quad [\text{S22}]$$

$$= \frac{t_{\text{h}}(1 - e^{-t/t_{\text{h}}}) - t_{\text{fp}}^{+}(1 + b\alpha)^{-1}(1 - e^{-t(1+b\alpha)/t_{\text{fp}}^{+}})}{t_{\text{h}}(1 + b\alpha) - t_{\text{fp}}^{+}},$$

$$\mathcal{P}_{\text{Tb}}^{-}(t) = \int_0^t dt' f_{\text{Tb}}^{-}(t') = b\alpha \mathcal{P}_{\text{Tb}}^{+}(t), \quad [\text{S23}]$$

where  $f_{\text{Tb}}^{\pm}(t)$  are the binding time distributions given by Eqs. S12 and S13. The expression for the average step is then

$$\langle \delta z(t) \rangle = (\mu_z + \Delta)(1 - \mathcal{P}_{\text{Tb}}^{+}(t) - \mathcal{P}_{\text{Tb}}^{-}(t))(1 - e^{-t/t_r}) + 2\Delta \mathcal{P}_{\text{Tb}}^{+}(t). \quad [\text{S24}]$$

The first term in Eq. S24 reflects the relaxation of the unbound subpopulation over a characteristic time  $t_r$  to the average position of the free end along the  $\hat{z}$  axis,  $\mu_z = \langle \hat{z} \cdot \mathbf{r} \rangle$ , where  $\mathbf{r}$  is the end-to-end vector of MyoV, and the average is taken over the equilibrium configurations of a two-legged polymer with one leg bound to the actin filament and the other leg free. As described in the next section, this average can be exactly derived and is related to the structural parameters of the system: the leg contour length  $L$ , the persistence length  $l_p$ , the strength of the end-tangent constraint  $\nu_c$  at the bound end, and the angle of the constraint direction  $\theta_c$  relative to the  $\hat{z}$  axis. The full expression for  $\mu_z$  is

$$\mu_z = l_p (1 - e^{-L/l_p}) (\coth \nu_c - \nu_c^{-1}) \cos \theta_c. \quad [\text{S25}]$$

For those interested in the derivation,  $\mu_z = \mu_{\parallel}^{\text{exact}} \cos \theta_c$ , where  $\mu_{\parallel}^{\text{exact}}$  is given by Eq. S38 below. The value of the polymer relaxation time is  $t_r \approx 5 \mu\text{s}$ , as discussed above. The second term in Eq. S24 is the contribution of trajectories that have bound to  $\mathbf{r}_{+}$ , and hence covered a distance of  $\delta z = 2\Delta$  along the filament axis. Trajectories binding to the initial site  $\mathbf{r}_{-}$  have  $\delta z = 0$ , and so do not appear in Eq. S24.

**Run Length and Velocity.** If the termination probability during each diffusive search is  $\mathcal{P}_t = 1 - \mathcal{P}_f - \mathcal{P}_{\text{Ts}} - \mathcal{P}_{\text{Ls}} - \mathcal{P}_b$ , then the mean number of searches during a run is  $\sum_{n=1}^{\infty} n(1 - \mathcal{P}_t)^{n-1} \mathcal{P}_t = 1/\mathcal{P}_t$ . The fraction of the searches within a run that leads to forward steps is  $\mathcal{P}_f/(1 - \mathcal{P}_t)$ , and the fraction that leads to backward steps is  $\mathcal{P}_b/(1 - \mathcal{P}_t)$ . The mean run length, assuming step size  $\Delta$ , is given by

$$z_{\text{run}} = \frac{\Delta(\mathcal{P}_f - \mathcal{P}_b)}{\mathcal{P}_t(1 - \mathcal{P}_t)} \approx \frac{\Delta t_{\text{dl}}(\alpha(g-1) + b(g-\alpha^2))}{(b+\alpha)(1+b\alpha)(t_{\text{Lb}} + g t_{\text{Tb}})}, \quad [\text{S26}]$$

where we have used the pathway probabilities from Eqs. S15, S16, S20, and S21 in the limit  $t_{\text{dl}} \gg t_{\text{Lb}}, t_{\text{Tb}}$ .

The mean velocity is  $v_{\text{run}} = z_{\text{run}}/t_{\text{run}}$ , where  $t_{\text{run}}$  is the average run time. To calculate the latter, we note that the mean waiting period (when both heads are bound to actin) is  $t_{\text{dl}} t_{\text{d2}}/(t_{\text{dl}} + t_{\text{d2}}) = g t_{\text{dl}}/(1+g)$ , whereas the mean binding times for the TH/LH are  $t_{\text{Tb}}$  (Eq. S14) and  $t_{\text{Lb}}$  (Eq. S19), respectively. Then,  $t_{\text{run}}$  for  $t_{\text{dl}} \gg t_{\text{Lb}}, t_{\text{Tb}}$  is given by

$$t_{\text{run}} = \frac{\mathcal{P}_f + \mathcal{P}_{\text{Ts}}}{\mathcal{P}_t(1 - \mathcal{P}_t)} \left( \frac{g}{1+g} t_{\text{dl}} + t_{\text{Tb}} \right) + \frac{\mathcal{P}_b + \mathcal{P}_{\text{Ls}}}{\mathcal{P}_t(1 - \mathcal{P}_t)} \left( \frac{g}{1+g} t_{\text{dl}} + t_{\text{Lb}} \right) \approx \frac{g t_{\text{dl}}^2}{t_{\text{Lb}} + g t_{\text{Tb}}}, \quad [\text{S27}]$$

where the first term is the contribution of steps involving TH detachment and the second term is the contribution of those involving LH detachment. The resulting expression for  $v_{\text{run}}$  is

$$v_{\text{run}} = \frac{z_{\text{run}}}{t_{\text{run}}} \approx \frac{\Delta}{t_{\text{dl}}} \left( \frac{1}{1+b\alpha} - \frac{\alpha}{g(b+\alpha)} \right). \quad [\text{S28}]$$

Eqs. S26–S28 are reproduced as Eq. 9.

### 3. Equilibrium Probability of Myosin End-Point Fluctuations

The equilibrium probability  $\mathcal{P}(\mathbf{r})$  of finding the MyoV free end at position  $\mathbf{r}$  (Fig. S3), needed to calculate  $t_{\text{fp}}^{+}$  in Eq. S9, can be obtained from calculating the end-to-end vector probabilities of the bound leg,  $\mathcal{P}_b(\mathbf{r}_b)$ , and the free leg,  $\mathcal{P}_f(\mathbf{r}_f)$ . Because  $\mathbf{r}$  is the sum of the end-to-end vectors of the legs,  $\mathbf{r} = \mathbf{r}_b + \mathbf{r}_f$ ,  $\mathcal{P}(\mathbf{r})$  can be written as a convolution of the two leg probabilities:

$$\mathcal{P}(\mathbf{r}) = \int d\mathbf{r}_b \int d\mathbf{r}_f \mathcal{P}_b(\mathbf{r}_b) \mathcal{P}_f(\mathbf{r}_f) \delta(\mathbf{r} - \mathbf{r}_b - \mathbf{r}_f). \quad [\text{S29}]$$

Each leg is an inextensible semiflexible polymer of contour length  $L$  and persistence length  $l_p$  (10), and one end of the bound leg is fixed at the origin  $\mathbf{r} = 0$ . The bound leg has two energetic contributions not present for the free leg: (i) the tangent vector of the bound leg at the origin,  $\hat{\mathbf{u}}_0$ , is subject to a harmonic constraint with energy  $\mathcal{H}_c = \frac{1}{2} k_B T \nu_c (\hat{\mathbf{u}}_0 - \hat{\mathbf{u}}_c)^2$ , where  $\nu_c$  and  $\hat{\mathbf{u}}_c$  are the strength and direction of the angle constraint, respectively ( $\hat{\mathbf{v}}$  denotes a unit vector, meaning  $|\hat{\mathbf{v}}| = 1$ ), and (ii) a load force  $\mathbf{F}$  is applied at the other end of the bound leg, where it joins the free leg. The force is oriented at an angle  $\theta_f$  clockwise from the  $-\hat{z}$  axis, as shown in Fig. S3. The axis  $\hat{z}$  is oriented from the minus to plus end of the actin filament. Both of these energetic contributions will lead to an overall tension in the bound leg that has to be accounted for in calculating the probability  $\mathcal{P}_b(\mathbf{r}_b)$ . In the following subsections, we present approximate analytical expressions for the leg probabilities  $\mathcal{P}_f(\mathbf{r}_f)$  and  $\mathcal{P}_b(\mathbf{r}_b)$ , justifying them by comparison with exact results for the first and second moments of the equilibrium probabilities. In the final subsection, we take the individual leg results and use Eq. S29 to derive a complete analytical expression for  $\mathcal{P}(\mathbf{r})$ , which is needed to calculate the first passage times (Eq. S9).

**Equilibrium End-to-End Probability of the Free Leg.** We start with the simpler case of the free leg, which is not under tension. There is no exact closed form analytical expression for the end-to-end vector probability  $\mathcal{P}_f(\mathbf{r}_f)$  of a semiflexible polymer [although the moments of the probability distribution are known analytically (10, 11), as illustrated below]. Mean field theory, however, provides an excellent approximation of the distribution (12):

$$\mathcal{P}_f(\mathbf{r}_f) = A_f \xi_f^{-9/2} \exp\left(-\frac{3\kappa}{4\xi_f}\right), \quad [\text{S30}]$$

where  $\kappa = L/l_p$  and  $\xi_f = 1 - r_f^2/L^2$ , and  $A_f$  is a normalization constant. The end-to-end vector  $\mathbf{r}_f$  can be specified by the polar and azimuthal angles  $\theta_f$  and  $\phi_f$ , as well as by the dimensionless radial variable  $\xi_f$ , which can only take on values between 0 and 1 for an inextensible polymer, because  $r_f \leq L$ . In this coordinate system, the normalization condition for the probability is



$$1 = \frac{L^3}{2} \int_0^1 d\xi_f (1 - \xi_f)^{1/2} \int_0^\pi d\theta_f \int_0^{2\pi} d\phi_f \mathcal{P}_f(\mathbf{r}_f). \quad [\text{S31}]$$

The normalization constant  $A_f$  is given by

$$A_f = \frac{9\sqrt{3}e^{3\kappa/4}\kappa^{7/2}}{8\pi^{3/2}L^3(3\kappa^2 + 12\kappa + 20)}. \quad [\text{S32}]$$

In the stiff limit of large persistence length ( $\kappa \rightarrow 0$ ), the probability in Eq. S30 goes to a delta function at  $r_f = L$ , as is appropriate for a rigid rod of  $L$ . In the opposite limit of flexible chains ( $\kappa \rightarrow \infty$ ), the probability goes to a Gaussian centered at  $\mathbf{r} = 0$ . Throughout the entire range of  $\kappa$ , the second moment of the probability distribution,  $\langle r_f^2 \rangle = 2L^2(3\kappa + 10)/(3\kappa^2 + 12\kappa + 20)$ , is within 1% of the exact result  $\langle r_f^2 \rangle_{\text{exact}} = 2L^2\kappa^{-2}(\kappa - 1 + e^{-\kappa})$  (10, 11). (The first moment  $\langle \mathbf{r}_f \rangle$  is trivially equal to zero in both the exact and approximate cases because of the radial symmetry of the distribution.) The approximation of Eq. S30 thus captures the physical features of the stiff and flexible limits and is reasonably accurate for our purposes.

### Equilibrium End-to-End Probability of the Bound Leg at Zero Load.

We first consider the bound leg in the absence of load on the joint ( $F = 0$ ). Our expression for  $\mathcal{P}_b(\mathbf{r}_b)$  should reduce to the free leg probability of Eq. S30 in the limit of zero constraint strength  $\nu_c = 0$ . For  $\nu_c \neq 0$ , we assume the effect of the end-tangent constraint can be approximated by the following ansatz:

$$\mathcal{P}_b(\mathbf{r}_b) = A_b \xi_b^{-9/2} \exp\left(-\frac{3\kappa}{4\xi_b} + \mathcal{T} \hat{\mathbf{u}}_c \cdot \hat{\mathbf{r}}_b\right), \quad [\text{S33}]$$

where  $\xi_b = 1 - r_b^2/L^2$ ,  $A_b$  is a normalization constant, and  $\mathcal{T}$  is an unknown function of  $\nu_c$  to be determined later, satisfying  $\mathcal{T} = 0$  at  $\nu_c = 0$ . Eq. S33 is identical in form to Eq. S30, except for the additional  $\mathcal{T}$  term in the exponential, which acts as an effective tension along  $\hat{\mathbf{u}}_c$  due to the end-tangent constraint. The normalization constant  $A_b$  is given by

$$A_b = A_f \frac{\mathcal{T}}{\sinh \mathcal{T}}. \quad [\text{S34}]$$

We choose  $\mathcal{T}$  so that the first and second moments of the probability distribution of Eq. S33 closely agree with the exact values for a semiflexible polymer under a harmonic end-tangent constraint. Because the analytical expressions for these exact values are not available in the literature, we derive them in the following way. We start by noting that the bound leg end-to-end vector  $\mathbf{r}_b = \int_0^L ds \hat{\mathbf{u}}(s)$ , where  $\hat{\mathbf{u}}(s) = d\mathbf{r}(s)/ds$  is the tangent vector at position  $s$  along the bound leg chain contour  $\mathbf{r}(s)$ ,  $0 \leq s \leq L$ . The tangent vectors for an inextensible chain all have unit length. The equilibrium statistics of  $\hat{\mathbf{u}}(s)$  for a semiflexible polymer are governed by the Green's function  $G(\hat{\mathbf{u}}, \hat{\mathbf{u}}'; s - s')$ , which describes the probability that a chain with tangent vector  $\hat{\mathbf{u}}(s) = \hat{\mathbf{u}}$  will have tangent vector  $\hat{\mathbf{u}}(s') = \hat{\mathbf{u}}'$  at some position  $s' \geq s$ . This Green's function has an exact spherical harmonic expansion of the form (11)

$$G(\hat{\mathbf{u}}, \hat{\mathbf{u}}'; s - s') = \sum_{lm} e^{-\frac{l(l+1)}{2\kappa}(s-s')} Y_{lm}^*(\hat{\mathbf{u}}) Y_{lm}(\hat{\mathbf{u}}'). \quad [\text{S35}]$$

For the initial tangent vector  $\hat{\mathbf{u}}_0 \equiv \hat{\mathbf{u}}(0)$  at  $s = 0$ , where the bound leg is attached to the actin, the harmonic constraint leads to a probability distribution  $\mathcal{P}_c(\hat{\mathbf{u}}_0)$  given by

$$\begin{aligned} \mathcal{P}_c(\hat{\mathbf{u}}_0) &= \frac{\nu_c}{2\pi(1 - e^{-2\nu_c})} \exp\left(-\frac{\nu_c}{2}(\hat{\mathbf{u}}_0 - \hat{\mathbf{u}}_c)^2\right) \\ &= \frac{\sqrt{\pi\nu_c}}{2} \frac{1}{\sinh \nu_c} \sum_{lm} I_{l+1/2}(\nu_c) Y_{lm}^*(\hat{\mathbf{u}}_c) Y_{lm}(\hat{\mathbf{u}}_0). \end{aligned} \quad [\text{S36}]$$

In the first line, the prefactor in front of the exponential is a normalization constant. In the second line, we have rewritten the exponential in a spherical harmonic expansion (13) involving modified spherical Bessel functions of the first kind  $I_\nu(x)$ . This form will facilitate carrying out the moment integrals below.

Let  $\hat{\mathbf{t}}$  be one of the three orthogonal unit vectors  $\hat{\mathbf{u}}_c$ ,  $\hat{\mathbf{v}}_c$ , or  $\hat{\mathbf{w}}_c$ , as defined in Fig. S3. These axes, with  $\hat{\mathbf{u}}_c$  being the constraint direction, are the easiest to work with for moment calculations. Using the definitions of  $G(\hat{\mathbf{u}}, \hat{\mathbf{u}}'; s - s')$  and  $\mathcal{P}_c(\hat{\mathbf{u}}_0)$  above, the first- and second-order moments with respect to one of the axes  $\hat{\mathbf{t}}$  can be written as

$$\begin{aligned} \langle \hat{\mathbf{t}} \cdot \mathbf{r}_b \rangle_{\text{exact}} &= \left\langle \int_0^L ds \hat{\mathbf{t}} \cdot \hat{\mathbf{u}}(s) \right\rangle_{\text{exact}} \\ &= \int_0^L ds \int d\hat{\mathbf{u}}_0 \int d\hat{\mathbf{u}} \mathcal{P}_c(\hat{\mathbf{u}}_0) G(\hat{\mathbf{u}}_0, \hat{\mathbf{u}}'; s) \hat{\mathbf{t}} \cdot \hat{\mathbf{u}}, \\ \langle (\hat{\mathbf{t}} \cdot \mathbf{r}_b)^2 \rangle_{\text{exact}} &= \left\langle \int_0^L ds \int_0^L ds' \hat{\mathbf{t}} \cdot \hat{\mathbf{u}}(s) \hat{\mathbf{t}} \cdot \hat{\mathbf{u}}(s') \right\rangle_{\text{exact}} \\ &= 2 \int_0^L ds \int_s^L ds' \int d\hat{\mathbf{u}}_0 \int d\hat{\mathbf{u}} \int d\hat{\mathbf{u}}' \\ &\quad \mathcal{P}_c(\hat{\mathbf{u}}_0) G(\hat{\mathbf{u}}_0, \hat{\mathbf{u}}; s) \hat{\mathbf{t}} \cdot \hat{\mathbf{u}} G(\hat{\mathbf{u}}, \hat{\mathbf{u}}'; s' - s) \hat{\mathbf{t}} \cdot \hat{\mathbf{u}}'. \end{aligned} \quad [\text{S37}]$$

By using Eqs. S35 and S36 and the properties of spherical harmonics, the integrals in Eq. S37 can be carried out exactly to yield the moments for any axis  $\hat{\mathbf{t}}$ . Let us define the average end-to-end component parallel to the constraint direction,  $\mu_{\parallel}^{\text{exact}} \equiv \langle \hat{\mathbf{u}}_c \cdot \mathbf{r}_b \rangle_{\text{exact}}$  (the first moments along  $\hat{\mathbf{v}}_c$  and  $\hat{\mathbf{w}}_c$  are zero). Similarly, define the parallel and perpendicular end-to-end SDs,  $\sigma_{\parallel}^{\text{exact}} \equiv \left( \langle (\hat{\mathbf{u}}_c \cdot \mathbf{r}_b)^2 \rangle_{\text{exact}} - \langle \hat{\mathbf{u}}_c \cdot \mathbf{r}_b \rangle_{\text{exact}}^2 \right)^{1/2}$  and  $\sigma_{\perp}^{\text{exact}} \equiv \left( \langle (\hat{\mathbf{v}}_c \cdot \mathbf{r}_b)^2 \rangle_{\text{exact}} \right)^{1/2} = \left( \langle (\hat{\mathbf{w}}_c \cdot \mathbf{r}_b)^2 \rangle_{\text{exact}} \right)^{1/2}$ . The results for these three quantities are

$$\begin{aligned} \mu_{\parallel}^{\text{exact}} &= L\kappa^{-1}(1 - k)\mathcal{L}(\nu_c), \\ \sigma_{\parallel}^{\text{exact}} &= \frac{L\kappa^{-1}}{3} \left( 2(3\kappa + k^3 - 1) - 9(k - 1)^2 \mathcal{L}^2(\nu_c) - \frac{6(k + 2)(k - 1)^2 \mathcal{L}(\nu_c)}{\nu_c} \right)^{1/2}, \\ \sigma_{\perp}^{\text{exact}} &= \frac{L\kappa^{-1}}{3} \left( 6\kappa - k^3 + 9k - 8 + \frac{3(k^3 - 3k + 2)\mathcal{L}(\nu_c)}{\nu_c} \right)^{1/2}, \end{aligned} \quad [\text{S38}]$$

where  $k \equiv \exp(-\kappa)$  and  $\mathcal{L}(\nu_c) \equiv \coth \nu_c - \nu_c^{-1}$  is the Langevin function.

The corresponding moments calculated from the probability distribution in Eq. S33 are

$$\begin{aligned} \mu_{\parallel} &= \frac{LL(\mathcal{T})}{\sqrt{\pi} \left( \frac{9}{4}\kappa(\kappa + 4) + 15 \right)} \left( \frac{3\sqrt{\pi}(10 - 3\kappa)}{2k^{3/4}} \operatorname{erfc} \frac{\sqrt{3\kappa}}{2} + 3\sqrt{3\kappa}(\kappa + 5) \right), \\ \sigma_{\parallel} &= L\kappa^{-1} \left( \frac{2\kappa^2(3\kappa + 10)(\mathcal{T} - 2\mathcal{L}(\mathcal{T}))}{(3\kappa(\kappa + 4) + 20)\mathcal{T}} - \frac{\mu_{\parallel}^2}{L^2} \right)^{1/2}, \\ \sigma_{\perp} &= L\kappa^{-1} \left( \frac{2\kappa^2(3\kappa + 10)\mathcal{L}(\mathcal{T})}{(3\kappa(\kappa + 4) + 20)\mathcal{T}} \right)^{1/2}. \end{aligned} \quad [\text{S39}]$$

To determine  $\mathcal{T}$ , we will set  $\mu_{\parallel}$  from Eq. S39 equal to  $\mu_{\parallel}^{\text{exact}}$  from Eq. S38. The resulting expression for  $\mathcal{T}$  is

$$T = \mathcal{L}^{-1} \left( \frac{\sqrt{\pi}(3\kappa(\kappa+4)+20)(1-k)k^{3/4}\mathcal{L}(\nu_c)}{2\kappa \left( \sqrt{\pi}(10-3\kappa)\operatorname{erfc}\left(\frac{\sqrt{3\kappa}}{2}\right) + 2\sqrt{3\kappa}(\kappa+5)k^{3/4} \right)} \right). \quad [\text{S40}]$$

$$\begin{aligned} \mathcal{P}(\mathbf{r}) &= A_f A_b \int d\mathbf{r}_b \int d\mathbf{r}_f \xi_f^{-9/2} \xi_b^{-9/2} \exp\left(-\frac{3\kappa}{4\xi_f} - \frac{3\kappa}{4\xi_b} + T' \hat{\mathbf{u}}_c \cdot \hat{\mathbf{r}}_b\right) \delta(\mathbf{r} - \mathbf{r}_b - \mathbf{r}_f) \\ &= A_f A_b \int d\mathbf{r}_b \xi_f^{-9/2} \xi_b^{-9/2} \exp\left(-\frac{3\kappa}{4\xi_f} - \frac{3\kappa}{4\xi_b} + T' \hat{\mathbf{u}}_c \cdot \hat{\mathbf{r}}_b\right). \end{aligned} \quad [\text{S46}]$$

Because the inverse Langevin function  $\mathcal{L}^{-1}(x)$  cannot be expressed analytically, for the purposes of evaluation, we use the Padé approximation  $\mathcal{L}^{-1}(x) \approx x(3-x^2)/(1-x^2)$  (14). For the parameter regimes  $\kappa \ll 1$  (large stiffness) and  $\nu_c \gg 1$  (strong end-tangent constraint), relevant to MyoV dynamics, Eq. S40 can be further simplified to yield

$$T \approx 1 + \frac{20\nu_c}{20 + 7\kappa\nu_c}. \quad [\text{S41}]$$

Eqs. S33, S34, and S41 completely describe the end-to-end vector probability distribution for the bound leg at zero load. By construction, the  $T$  of Eq. S41 leads to a  $\mu_{\parallel}$  that closely agrees with the exact value  $\mu_{\parallel}^{\text{exact}}$  from Eq. S38. In addition, the other moments are reproduced well by the approximate probability distribution, as shown in Fig. S4. The exact and approximate values differ by no more than 7% over the entire parameter range of  $l_p$  and  $\nu_c$ . This range covers the most likely parameters for MyoV dynamics, as discussed in the main text.

#### Equilibrium End-to-End Probability of the Bound Leg Under Load.

In the presence of a load force  $\mathbf{F}$ , the probability distribution in Eq. S33 is multiplied by a factor of  $\exp(\beta F r_b \hat{\mathbf{F}} \cdot \hat{\mathbf{r}}_b) = \exp(\beta FL(1-\xi_b)^{1/2} \hat{\mathbf{F}} \cdot \hat{\mathbf{r}}_b)$ . In the stiff limit  $\kappa \ll 1$ , the main contributions to the end-to-end vector probability are for  $\xi_b \ll 1$ , because  $r_b$  approaches  $L$ , the leg contour length. Thus, the contribution of the load can be approximated as  $\exp(\beta FL \hat{\mathbf{F}} \cdot \hat{\mathbf{r}}_b)$ . With this approximation, the overall form of Eqs. S33 and S34 is preserved under load, with the substitutions  $T \rightarrow T'$  and  $\hat{\mathbf{u}}_c \rightarrow \hat{\mathbf{u}}_c'$ . The probability distribution becomes

$$\mathcal{P}_b(\mathbf{r}_b) = A_b \xi_b^{-9/2} \exp\left(-\frac{3\kappa}{4\xi_b} + T' \hat{\mathbf{u}}_c' \cdot \hat{\mathbf{r}}_b\right), \quad [\text{S42}]$$

$$A_b = A_f \frac{T'}{\sinh T'}, \quad [\text{S43}]$$

where the new effective tension along the leg, written in terms of its  $\hat{\mathbf{x}}$  and  $\hat{\mathbf{z}}$  components, is

$$\begin{aligned} T' &= \sqrt{(T'_x)^2 + (T'_z)^2}, \quad T'_x = T \sin \theta_c + \beta FL \sin \theta_F, \\ T'_z &= T \cos \theta_c - \beta FL \cos \theta_F. \end{aligned} \quad [\text{S44}]$$

The new effective tension direction is  $\hat{\mathbf{u}}_c' = \sin \theta_c' \hat{\mathbf{x}} + \cos \theta_c' \hat{\mathbf{z}}$ , which is oriented at an angle  $\theta_c'$  from the  $\hat{\mathbf{z}}$  axis:

$$\theta_c' = \theta_c + \tan^{-1} \left( \frac{\beta FL \sin(\theta_c + \theta_F)}{T - \beta FL \cos(\theta_c + \theta_F)} \right). \quad [\text{S45}]$$

**Combining the Individual Leg Probabilities to Find the Total End-to-End Vector Probability Distribution.** The final step in the derivation of  $\mathcal{P}(\mathbf{r})$  is to evaluate Eq. S29. Using  $\mathcal{P}_f$  from Eq. S30 and  $\mathcal{P}_b$  from Eq. S42, the convolution integral in Eq. S29 has the form

In the second step, we have carried out the integration over the free leg end-to-end vector  $\mathbf{r}_f$ , with the delta function making the radial variable  $\xi_f = 1 - r_f^2/L^2$  a function of  $\mathbf{r}$  and  $\mathbf{r}_b$ :

$$\xi_f = 1 - \frac{r^2 + r_b^2 - 2rr_b \cos \theta_b}{L^2}, \quad [\text{S47}]$$

where  $\theta_b$  is the angle between  $\mathbf{r}$  and  $\mathbf{r}_b$ . Because we are interested in probabilities of finding the free end of MyoV along the actin filament, let us confine the rest of the calculation to  $\mathbf{r} = z\hat{\mathbf{z}}$ , where  $-2L \leq z \leq 2L$  (because this is the maximum range that the two-legged structure of total contour length  $2L$  can access). The unit vector  $\hat{\mathbf{r}}_b$  can be represented in spherical coordinates by the polar and azimuthal angles  $(\theta_b, \phi_b)$ , and  $\hat{\mathbf{u}}_c'$  can be represented by  $(\theta_c', \phi_c' = 0)$ . Thus,

$$\hat{\mathbf{u}}_c' \cdot \hat{\mathbf{r}}_b = \cos \theta_b \cos \theta_c' + \cos \phi_b \sin \theta_b \sin \theta_c'. \quad [\text{S48}]$$

Writing the integration element in Eq. S46 as  $d\mathbf{r}_b = r_b^2 d\cos \theta_b d\phi_b$ , we can carry out the integral over  $\phi_b$  using Eq. S48. The result is

$$\begin{aligned} \mathcal{P}(z\hat{\mathbf{z}}) &= 2\pi A_f A_b \int_0^L r_b^2 dr_b \int_{-1}^1 d\cos \theta_b \xi_f^{-9/2} \xi_b^{-9/2} \\ &\times \exp\left(-\frac{3\kappa}{4\xi_f} - \frac{3\kappa}{4\xi_b} + T'_z \cos \theta_b\right) \cdot I_0(T'_x \sin \theta_b), \end{aligned} \quad [\text{S49}]$$

where  $I_0(x)$  is the zeroth-order modified Bessel function of the first kind. To simplify the integration, we will change variables from  $(r_b, \cos \theta_b)$  to  $(\xi_b, \xi_f)$ . From the definitions of  $\xi_b$  and  $\xi_f$ , and from Eq. S47, the two sets of variables are related by

$$r_b = L\sqrt{1-\xi_b}, \quad \cos \theta_b = \frac{z^2 + L^2(\xi_f - \xi_b)}{2zL\sqrt{1-\xi_b}}, \quad [\text{S50}]$$

leading to a Jacobian determinant  $|\det J| = L^2/(4|z|(1-\xi_b))$  for the change of variables. Using these relations, Eq. S49 becomes

$$\begin{aligned} \mathcal{P}(z\hat{\mathbf{z}}) &= \frac{L^4 \pi A_f A_b}{2|z|} \int_0^{u_b(z)} d\xi_b \int_0^{u_f(z, u_b)} d\xi_f \xi_f^{-9/2} \xi_b^{-9/2} \\ &\cdot \exp\left(-\frac{3\kappa}{4\xi_f} - \frac{3\kappa}{4\xi_b} + T'_z \frac{z^2 + L^2(\xi_f - \xi_b)}{2zL\sqrt{1-\xi_b}}\right) \\ &\cdot I_0\left(T'_x \sqrt{1 - \left(\frac{z^2 + L^2(\xi_f - \xi_b)}{2zL\sqrt{1-\xi_b}}\right)^2}\right), \end{aligned} \quad [\text{S51}]$$

where the upper limits of integration are given by

$$u_b(z) = \frac{2|z|}{L} - \frac{z^2}{L^2}, \quad u_f(z, \xi_b) = \xi_b + \frac{2|z|\sqrt{1-\xi_b}}{L} - \frac{z^2}{L^2}. \quad [\text{S52}]$$

In the stiff limit  $\kappa \rightarrow 0$ , the main contributions to the integral come from  $\xi_b \ll 1$  and  $\xi_f \ll 1$ . Additionally, the location of the binding sites we consider,  $|z| = 36$  nm, is comparable to the leg contour length  $L = 35$  nm. We can then approximately carry out the integral in Eq. S51 by replacing the integration limits  $u_b \rightarrow 1$  and  $u_f \rightarrow 1$ , and by substituting

$$\frac{z^2 + L^2(\xi_f - \xi_b)}{2zL\sqrt{1-\xi_b}} \rightarrow \frac{z}{2L}. \quad [\text{S53}]$$

With these approximations, the integral in Eq. S51 evaluates to

$$\mathcal{P}(z\hat{z}) \approx \frac{8\pi L^4 A_f A_b}{729\kappa^7 |z|} \left( 20\sqrt{3\pi} e^{3\kappa/4} \operatorname{erfc}\left(\frac{\sqrt{3\kappa}}{2}\right) + 3\sqrt{\kappa}(\kappa(3\kappa+10)+20) \right)^2 \cdot I_0 \left( T'_x \sqrt{1 - \frac{z^2}{4L^2}} \right) e^{\frac{1}{2} \left( \frac{T'_x}{L} - 3\kappa \right)}. \quad [\text{S54}]$$

Upon substituting in Eq. S32 for  $A_f$  and in Eq. S43 for  $A_b$ , and upon expanding  $\mathcal{P}(z\hat{z})$  up to the second order in  $\kappa$ , we get the final simplified form of the probability. The result evaluated at  $z = \pm \Delta$  is given by Eq. 20:

$$\mathcal{P}(\mathbf{r}_{\pm}) \approx \frac{(3\kappa(7\kappa+20)+200)T'}{1,600\pi L^2 \Delta \sinh T'} I_0 \left( T'_x \sqrt{1 - \frac{\Delta^2}{4L^2}} \right) e^{\pm \frac{T'_x \Delta}{2L}}. \quad [\text{S55}]$$

Together with Eq. S41 for  $\mathcal{T}$  and Eq. S44 for  $\mathcal{T}'$ , we now have a complete analytical expression for the probability distribution of the MyoV free end at any location along the actin filament axis.

An analogous approach can be used to find  $\mathcal{P}(\mathbf{r})$  analytically at any  $\mathbf{r}$  and not just along  $\hat{z}$ . The resulting 3D probability distribution allows us to generate sample diffusive trajectories for the end-to-end vector  $\mathbf{r}$  in various MyoV kinetic pathways,

as shown in Fig. S5. These are numerical solutions to the Fokker–Planck equation (15) for diffusion along an energy surface  $U(\mathbf{r}) = -k_B T \log \mathcal{P}(\mathbf{r})$  with head diffusivity  $D_h$ .

#### 4. Stall Force

Based on the earlier results for the step probabilities and first passage times, one can derive a simple expression for the stall force  $F_{\text{stall}}$ , defined by the condition that backward and forward step probabilities are equal,  $\mathcal{P}_f = \mathcal{P}_b$  at  $F = F_{\text{stall}}$ . From Eqs. S15 and S21, the ratio of the two probabilities is

$$\frac{\mathcal{P}_b}{\mathcal{P}_f} = \frac{\alpha(1+b\alpha)(t_{d1}+t_h)(t_{d1}+t_{Tb}-t_h)}{g(b+\alpha)t_{d1}(t_{d1}+t_{Lb})} \approx g^{-1} \frac{\alpha(1+b\alpha)}{b+\alpha}. \quad [\text{S56}]$$

The approximation in the second line is valid when  $t_{d1} \gg t_{Tb}, t_{Lb}$ , which is typically the case.

Setting the right-hand side of Eq. S56 equal to 1, we can solve for the value  $\alpha = \alpha_{\text{stall}}$  at the stall force:

$$\alpha_{\text{stall}} = \frac{g-1 + \sqrt{(g-1)^2 + 4gb^2}}{2b}. \quad [\text{S57}]$$

Using Eq. S9 for  $t_{fp}^{\pm}$ , Eq. S55 for the equilibrium free end probability  $\mathcal{P}(\mathbf{r}_{\pm})$ , and the definition of  $\mathcal{T}'$  from Eq. S44, we can rewrite Eq. S57 as follows:

$$\frac{g-1 + \sqrt{(g-1)^2 + 4gb^2}}{2b} = \alpha_{\text{stall}} = \frac{\mathcal{P}(\mathbf{r}_-)}{\mathcal{P}(\mathbf{r}_+)} \Bigg|_{F=F_{\text{stall}}} = \exp \left( -\frac{\Delta T}{L} \cos \theta_c + \beta \Delta F_{\text{stall}} \cos \theta_F \right). \quad [\text{S58}]$$

This equation can be directly solved for  $F_{\text{stall}}$ , giving Eq. 10:

$$F_{\text{stall}} = \frac{k_B T}{\cos \theta_F} \left( \frac{T}{L} \cos \theta_c + \frac{1}{\Delta} \log \frac{g-1 + \sqrt{(g-1)^2 + 4gb^2}}{2b} \right). \quad [\text{S59}]$$

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