Supplementary Information

How kinesin waits for ATP affects the nucleotide and load dependence of the stepping kinetics

R. Takaki

Department of Physics, The University of Texas at Austin, Austin, TX 78712

M. L. Mugnai, Y. Goldtzvik, and D. Thirumalai

Department of Chemistry, The University of Texas at Austin, Austin, TX 78712

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CONTENTS

I. DERIVATION OF THE RUN LENGTH DISTRIBUTION

The summation in Eq.(4) in the main text,

$$P(n) = \sum_{m,l=0}^{\infty} \frac{(m+l)!}{m!l!} \Big(\frac{J^+}{J_T}\Big)^m \Big(\frac{J^-}{J_T}\Big)^l \Big(\frac{J^\gamma}{J_T}\Big) \delta_{m-l,n}, \tag{S1}$$

can be carried out for $n > 0$, leading to,

$$\begin{aligned} P(n>0) &= \Big(\frac{J^+}{J_T}\Big)^n \Big(\frac{J^\gamma}{J_T}\Big) \sum_{l=0}^{\infty} \Big(\frac{J^+J^-}{J_T^2}\Big)^l \frac{(2l+n)}{(n+l)!l!} \\ &= \Big(\frac{J^+}{J_T}\Big)^n \Big(\frac{J^\gamma}{J_T}\Big) \, {}_2\mathrm{F}_1\Big(\frac{1+n}{2}, \frac{2+n}{2}; 1+n; 4\frac{J^+J^-}{J_T^2}\Big), \end{aligned} \tag{S2}$$

where ${}_2\mathrm{F}_1$ is Gaussian hypergeometric function. By using the special case of ${}_2\mathrm{F}_1$ (page 556 of [1]), we obtain the following expression for the run length distribution,

$$P(n \gtrless 0) = \Big(\frac{2J^\pm}{J_T + \sqrt{J_T^2 - 4J^+J^-}}\Big)^{|n|} \frac{J^\gamma}{\sqrt{J_T^2 - 4J^+J^-}}. \tag{S3}$$

In the above equation n is non-dimensional quantity which denotes the position of the motor on the track. Notice that Eq.(S3) is independent of ATP concentration, which is in accord with experiments [2, 3] as long as ATP concentration exceeds $\approx 10\mu$M. At lower ATP concentration, the mean run length does moderately depend on [T]. This suggests that at very low [T] the motor head could enter the vulnerable 1HB state spontaneously. In such a state the LH could detach before ATP binds to it. This would occur if the binding time for ATP (inverse of a pseudo first order constant) is greater than the detachment time.

II. DERIVATION OF THE VELOCITY DISTRIBUTION

In our model (Fig.1(c) and (d) in the main text), the probability distribution of forward step, backward step, and detachment at time t, and the corresponding Laplace transform

($\mathcal{L}(f) = \int_0^\infty \mathrm{e}^{-st} f(t)dt$) are given by the following expressions,

$$\begin{aligned} f_+(t) &= \int_0^t dt' k\mathrm{e}^{-kt'} k^+ \mathrm{e}^{-(k^+ + k^- + \gamma)(t-t')} \\ &= \frac{kk^+}{k^+ + k^- + \gamma - k}(\mathrm{e}^{-kt} - \mathrm{e}^{-(k^+ + k^- + \gamma)t}) \\ \tilde{f}_+(s) &= \frac{kk^+}{(s+k)(s+k^+ + k^- + \gamma)}; \end{aligned} \tag{S4}$$

and

$$\begin{aligned} f_-(t) &= \int_0^t dt' k\mathrm{e}^{-kt'} k^- \mathrm{e}^{-(k^+ + k^- + \gamma)(t-t')} \\ &= \frac{kk^-}{k^+ + k^- + \gamma - k}(\mathrm{e}^{-kt} - \mathrm{e}^{-(k^+ + k^- + \gamma)t}) \\ \tilde{f}_-(s) &= \frac{kk^-}{(s+k)(s+k^+ + k^- + \gamma)}; \end{aligned} \tag{S5}$$

$$\begin{aligned} f_\gamma(t) &= \int_0^t dt' k\mathrm{e}^{-kt'} \gamma \mathrm{e}^{-(k^+ + k^- + \gamma)(t-t')} \\ &= \frac{k\gamma}{k^+ + k^- + \gamma - k}(\mathrm{e}^{-kt} - \mathrm{e}^{-(k^+ + k^- + \gamma)t}) \\ \tilde{f}_\gamma(s) &= \frac{k\gamma}{(s+k)(s+k^+ + k^- + \gamma)}. \end{aligned} \tag{S6}$$

The probability that the motor takes m forward steps and l backward steps before detaching at time t can be written as,

$$\begin{aligned} f(m,l,t) = \frac{(m+l)!}{m!l!} &\int_0^t dt_{m+l} \int_0^{t_{m+l}} dt_{m+l-1} \cdots \int_0^{t_3} dt_2 \int_0^{t_2} dt_1 \\ &\prod_{i=1}^{m} f_+(t_i - t_{i-1}) \prod_{i=m+1}^{m+l} f_-(t_i - t_{i-1}) f_\gamma(t - t_{m+l}). \end{aligned} \tag{S7}$$

The factorial term in Eq.(S7) accounts for the number of ways in which the motor can take m forward steps and l backward steps for a net displacement of $m - l$. In Laplace space, Eq.(S7) is a multiplication of all the dwell time distributions up to the time of detachment,

$$\begin{aligned}\tilde{f}(m,l,s) =& \frac{(m+l)!}{m!l!}(\tilde{f}_+(s))^m(\tilde{f}_-(s))^l\tilde{f}_\gamma(s) \\ =& \frac{(m+l)!}{m!l!}k^{m+l+1}(k^+)^m(k^-)^l\gamma\frac{1}{(s+k)^{m+l+1}(s+k^++k^-+\gamma)^{m+l+1}}.\end{aligned} \tag{S8}$$

In order to obtain $f(m,l,t)$ by inverse Laplace transform, we need to evaluate the following integral,

$$\frac{1}{2\pi i}\int_{c-i\infty}^{c+i\infty}\frac{1}{(s+\xi_1)^{n+1}(s+\xi_2)^{n+1}}\mathrm{e}^{st}ds, \tag{S9}$$

where we set $m+l=n$, $\xi_1=k$, and $\xi_2=k^++k^-+\gamma$. This can be done using the standard residue theorem. For the generic case of $\xi_1\neq\xi_2$, there are two distinct poles of order $n+1$.

$$\begin{aligned}\mathrm{Res}\Big[\frac{1}{(s+\xi_1)^{n+1}(s+\xi_2)^{n+1}}\mathrm{e}^{st}\Big]\Big|_{s=-\xi_1} &= \frac{1}{n!}\frac{d^n}{ds^n}\Big[\frac{1}{(s+\xi_2)^{n+1}}\mathrm{e}^{st}\Big]\Big|_{s=-\xi_1} \\ &= \frac{1}{n!}\sum_{p=0}^{n}(-1)^p\binom{n}{p}\frac{t^{n-p}\mathrm{e}^{-\xi_1 t}}{(-\xi_1+\xi_2)^{n+p+1}}\frac{(n+p)!}{n!},\end{aligned} \tag{S10}$$

$$\begin{aligned}\mathrm{Res}\Big[\frac{1}{(s+\xi_1)^{n+1}(s+\xi_2)^{n+1}}\mathrm{e}^{st}\Big]\Big|_{s=-\xi_2} &= \frac{1}{n!}\frac{d^n}{ds^n}\Big[\frac{1}{(s+\xi_1)^{n+1}}\mathrm{e}^{st}\Big]\Big|_{s=-\xi_2} \\ &= \frac{1}{n!}\sum_{p=0}^{n}(-1)^p\binom{n}{p}\frac{t^{n-p}\mathrm{e}^{-\xi_2 t}}{(-\xi_2+\xi_1)^{n+p+1}}\frac{(n+p)!}{n!}.\end{aligned} \tag{S11}$$

Thus, by combining Eqs.(10.47.9), (10.49.1), and (10.49.12) of the NIST handbook of Mathematics [4], the inverse Laplace transform in Eq.(S9) becomes,

$$\begin{aligned}(S9) =& \frac{1}{n!}\sum_{p=0}^{n}(-1)^p\binom{n}{p}\frac{t^{n-p}\mathrm{e}^{-\xi_1 t}}{(-\xi_1+\xi_2)^{n+p+1}}\frac{(n+p)!}{n!}+\frac{1}{n!}\sum_{p=0}^{n}(-1)^p\binom{n}{p}\frac{t^{n-p}\mathrm{e}^{-\xi_2 t}}{(-\xi_2+\xi_1)^{n+p+1}}\frac{(n+p)!}{n!} \\ =& \frac{t^n}{\sqrt{\pi}n!}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}\frac{\sqrt{(\xi_1-\xi_2)t}}{(\xi_2-\xi_1)^{n+1}}K_{n+\frac{1}{2}}(\frac{\xi_1-\xi_2}{2}t)+\frac{t^n}{\sqrt{\pi}n!}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}\frac{\sqrt{(\xi_2-\xi_1)t}}{(\xi_1-\xi_2)^{n+1}}K_{n+\frac{1}{2}}(\frac{\xi_2-\xi_1}{2}t),\end{aligned} \tag{S12}$$

where K is the modified Bessel function of the second kind.

Hence, Eq.(S7) becomes,

$$f(m,l,t) = \frac{\gamma}{m!l!}\frac{1}{\sqrt{\pi}}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}t^{m+l}k^{m+l+1}(k^+)^m(k^-)^l\Big[\frac{\sqrt{(\xi_1-\xi_2)t}}{(\xi_2-\xi_1)^{m+l+1}}\ K_{m+l+\frac{1}{2}}(\frac{\xi_1-\xi_2}{2}t) + \frac{\sqrt{(\xi_2-\xi_1)t}}{(\xi_1-\xi_2)^{m+l+1}}\ K_{m+l+\frac{1}{2}}(\frac{\xi_2-\xi_1}{2}t)\Big]. \tag{S13}$$

We can simplify the above expression by employing the following identity for modified Bessel function given in Eq.(10.34.2) in [4], and rewrite it as,

$$K_{n+\frac{1}{2}}(-x) = -i\big(\pi I_{n+\frac{1}{2}}(x) + (-1)^n K_{n+\frac{1}{2}}(x)\big) \qquad (x>0), \tag{S14}$$

where I is the modified Bessel function of the first kind. Therefore, we further simplify Eq.(S13) as follows.

For $\xi_1-\xi_2>0$,

$$f(m,l,t) = \frac{\gamma}{m!l!}\sqrt{\pi}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}t^{m+l}\frac{k^{m+l+1}(k^+)^m(k^-)^l}{(\xi_1-\xi_2)^{m+l+1}}\sqrt{(\xi_1-\xi_2)t}\ I_{m+l+\frac{1}{2}}(\frac{\xi_1-\xi_2}{2}t). \tag{S15}$$

If $\xi_2-\xi_1>0$ then,

$$f(m,l,t) = \frac{\gamma}{m!l!}\sqrt{\pi}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}t^{m+l}\frac{k^{m+l+1}(k^+)^m(k^-)^l}{(\xi_2-\xi_1)^{m+l+1}}\sqrt{(\xi_2-\xi_1)t}\ I_{m+l+\frac{1}{2}}(\frac{\xi_2-\xi_1}{2}t). \tag{S16}$$

Both cases are written as,

$$f(m,l,t) = \frac{\gamma\sqrt{\pi}}{m!l!}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t}t^{m+l}\frac{k^{m+l+1}(k^+)^m(k^-)^l}{|\xi_2-\xi_1|^{m+l+1}}\sqrt{|\xi_2-\xi_1|t}\ I_{m+l+\frac{1}{2}}(\frac{|\xi_2-\xi_1|}{2}t). \tag{S17}$$

We finally obtain the expression for the velocity distribution.

For $m-l>0$,

$$P(v>0)=\sum_{\substack{m,l\\m>l}}^{\infty}\int_0^{\infty} f(m,l,t)\delta(v-\frac{m-l}{t})dt$$
$$=\sum_{\substack{m,l\\m>l}}^{\infty}\frac{m-l}{v^2}\frac{\gamma\sqrt{\pi}}{m!l!}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}\frac{m-l}{v}}(\frac{m-l}{v})^{m+l+\frac{1}{2}}\frac{k^{m+l+1}(k^+)^m(k^-)^l}{|\xi_2-\xi_1|^{m+l+\frac{1}{2}}}\ I_{m+l+\frac{1}{2}}(\frac{|\xi_2-\xi_1|}{2}\frac{m-l}{v}). \tag{S18}$$

For $m-l<0$,

$$P(v<0)=\sum_{\substack{m,l\\l>m}}^{\infty}\int_0^{\infty} f(m,l,t)\delta(v-\frac{m-l}{t})dt$$
$$=\sum_{\substack{m,l\\l>m}}^{\infty}\frac{l-m}{v^2}\frac{\gamma\sqrt{\pi}}{m!l!}\mathrm{e}^{-\frac{\xi_1+\xi_2}{2}\frac{m-l}{v}}(\frac{m-l}{v})^{m+l+\frac{1}{2}}\frac{k^{m+l+1}(k^+)^m(k^-)^l}{|\xi_2-\xi_1|^{m+l+\frac{1}{2}}}\ I_{m+l+\frac{1}{2}}(\frac{|\xi_2-\xi_1|}{2}\frac{m-l}{v}). \tag{S19}$$

Where $\xi_1=k$ and $\xi_2=k^++k^-+\gamma$.

Let us now consider the case $\xi_1=\xi_2$. Let $\xi_1=\xi_2\equiv\xi$, then we need to evaluate the following integral,

$$\frac{1}{2\pi i}\int_{c-i\infty}^{c+i\infty}\frac{1}{(s+\xi)^{2(n+1)}}\mathrm{e}^{st}ds. \tag{S20}$$

In this case, we have a single pole of order $2(n+1)$. The result for $f(m,l,t)$ becomes,

$$f(m,l,t)=\frac{(m+l)!}{m!l!}k^{m+l+1}(k^+)^m(k^-)^l\gamma\frac{t^{2(m+l)+1}\mathrm{e}^{-\xi t}}{(2(m+l)+1)!}, \tag{S21}$$

leading to

$$P(v>0)=\sum_{\substack{m,l\\m>l}}^{\infty}\frac{m-l}{v^2}\frac{(m+l)!}{m!l!}\frac{k^{m+l+1}(k^+)^m(k^-)^l\gamma}{(2(m+l)+1)!}\Big(\frac{m-l}{v}\Big)^{2(m+l)+1}\mathrm{e}^{-\xi\frac{m-l}{v}}, \tag{S22}$$

$$P(v<0)=\sum_{\substack{m,l\\ l>m}}^{\infty}\frac{l-m}{v^2}\frac{(m+l)!}{m!l!}\frac{k^{m+l+1}(k^+)^m(k^-)^l\gamma}{(2(m+l)+1)!}\Big(\frac{m-l}{v}\Big)^{2(m+l)+1}\mathrm{e}^{-\xi\frac{m-l}{v}}. \tag{S23}$$

Note that Eqs.(S21)-(S23) could also be obtained by taking the limit for $\xi_1 \to \xi_2$ in Eqs.(S17)-(S19), knowing that the limit for small argument of the modified Bessel function of the first kind is given in Eq.(10.30.1) of [4].

For the kinetic scheme in Fig.1(c) and (d) in the main text, the expressions for the velocity distributions are identical. However, the rate k, k^+, k^-, and γ depend on ATP concentration in a different manner for the two models, which describe two distinct waiting states of kinesin for ATP.

III. RANDOMNESS PARAMETER

Randomness parameter, which in some sense is easier to measure in experiments, is a useful way to estimate the number of rate limiting steps of molecular motors [5, 6]. We here discuss two types of randomness parameters for our models in the main text, chemical randomness r_C and mechanical randomness r_M, which are connected by a relation denoted by $\bar{r}_C$ as shown below.

A. Chemical randomness parameter, r_C

Chemical randomness is given by the following expression,

$$r_C=\frac{\langle\tau^2\rangle-\langle\tau\rangle^2}{\langle\tau\rangle^2}, \tag{S24}$$

where τ is the dwell time of the motor at a given site and the bracket denotes an average over an ensemble of motors. Dwell time distributions for stepping forward (+) or backward (−) at a site at time t in our models [Fig.1(c) and (d) in the main text] are given by,

$$\begin{aligned}f_{\pm}(t)&=\int_0^t dt' k\mathrm{e}^{-kt'}k^{\pm}\mathrm{e}^{-(k^++k^-+\gamma)(t-t')}\\&=\frac{kk^{\pm}}{k^++k^-+\gamma-k}(\mathrm{e}^{-kt}-\mathrm{e}^{-(k^++k^-+\gamma)t}).\end{aligned} \tag{S25}$$

Thus, the first and second moment of dwell time distribution conditioned by forward or backward step are,

$$\langle\tau_{\pm}\rangle = \frac{\int_0^\infty \tau_{\pm} f_{\pm}(\tau_{\pm}) d\tau_{\pm}}{\int_0^\infty f_{\pm}(\tau_{\pm}) d\tau_{\pm}} = \frac{k + k^+ + k^- + \gamma}{k(k^+ + k^- + \gamma)}, \tag{S26}$$

$$\begin{aligned} \langle\tau_{\pm}^2\rangle =& \frac{\int_0^\infty \tau_{\pm}^2 f_{\pm}(\tau_{\pm}) d\tau_{+}}{\int_0^\infty f_{\pm}(\tau_{\pm}) d\tau_{\pm}} \\ =& 2\frac{k^2 + k(k^+ + k^- + \gamma) + (k^+ + k^- + \gamma)^2}{k^2(k^+ + k^- + \gamma)^2}. \end{aligned} \tag{S27}$$

Thus, $(\langle\tau_+^2\rangle - \langle\tau_+\rangle^2)/\langle\tau_+\rangle^2 = (\langle\tau_-^2\rangle - \langle\tau_-\rangle^2)/\langle\tau_-\rangle^2$, which allows us to express r_C as

$$r_C = \frac{k^2 + (k^+ + k^- + \gamma)^2}{(k + k^+ + k^- + \gamma)^2}. \tag{S28}$$

B. Mechanical randomness parameter, r_M

We define r_M as,

$$r_M = \lim_{t\to\infty} \frac{\langle n^2(t)\rangle - \langle n(t)\rangle^2}{\langle n(t)\rangle}, \tag{S29}$$

where $n(t)$ is the position of the motor at time t. In our model, we can obtain the expression for the probability distribution that the motor is at site n at time t, which is needed to calculate the moments in (S29).

Using (S17) we obtain the probability that the motor takes $n = (m - l)$,

$$\begin{aligned} f(n,t) =& \sum_{m,l} \delta_{m,n+l} f(m,l,t) \\ =& \sum_{l=0}^{\infty} \frac{\gamma\sqrt{\pi}}{(n+2l)!l!} \mathrm{e}^{-\frac{\xi_1+\xi_2}{2}t} t^{n+2l+\frac{1}{2}} \frac{k^{n+2l+1}(k^+)^{n+l}(k^-)^l}{|\xi_2 - \xi_1|^{n+2l+\frac{1}{2}}} \; I_{n+2l+\frac{1}{2}}\left(\frac{|\xi_2-\xi_1|}{2}t\right). \end{aligned} \tag{S30}$$

However, as written the sum (S30) accounts for contributions from motors that have detached from the track before sufficiently long time t has elapsed. The appropriate probability distribution to get the moments in Eq.(S29) is the re-normalized probability distribution at each time t, which accounts only for the motors which stay on the track for a long time t. We denote this probability distribution as $\bar{f}$, which is defined as,

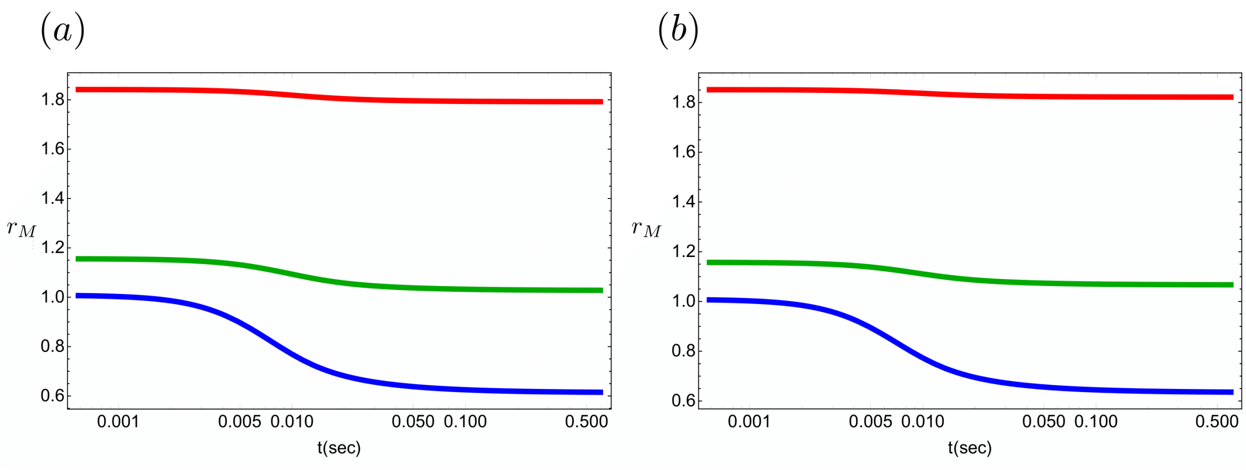

FIG. S1. Relaxation of mechanical randomness parameter at $F = 0$pN (blue), $F = 4$pN (green), and $F = 6$pN (red). [T]=1mM in all cases. (a) 2HB model. (b) 1HB model. In all cases $t = 0.5$s is long enough for r_M to have a plateau value.

$$\bar{f}(n,t) = \frac{1}{C}\sum_{l=0}^{\infty} \frac{\gamma\sqrt{\pi}}{(n+2l)!l!} e^{-\frac{\xi_1+\xi_2}{2}t} t^{n+2l+\frac{1}{2}} \frac{k^{n+2l+1}(k^+)^{n+l}(k^-)^l}{|\xi_2-\xi_1|^{n+2l+\frac{1}{2}}} \, I_{n+2l+\frac{1}{2}}\left(\frac{|\xi_2-\xi_1|}{2}t\right), \quad \text{(S31)}$$

where

$$C = \sum_{l=0}^{\infty}\sum_{n=-\infty}^{\infty} \frac{\gamma\sqrt{\pi}}{(n+2l)!l!} e^{-\frac{\xi_1+\xi_2}{2}t} t^{n+2l+\frac{1}{2}} \frac{k^{n+2l+1}(k^+)^{n+l}(k^-)^l}{|\xi_2-\xi_1|^{n+2l+\frac{1}{2}}} I_{n+2l+\frac{1}{2}}\left(\frac{|\xi_2-\xi_1|}{2}t\right). \quad \text{(S32)}$$

We used the distribution Eq.(S31) to obtain the first and second moment of n for the calculation of mechanical randomness parameter. In practice, we calculated the first and second moments $\langle n(t)\rangle$ and $\langle n^2(t)\rangle$ at $t = 0.5$s, which is long enough for $\langle n(t)\rangle$ to decay significantly. The double summation in Eq.(S32) should include enough terms to ensure convergence of $\langle n(t)\rangle$ and $\langle n^2(t)\rangle$. We truncated the summation at 30 and 130 for l and n, respectively. Needless to say that if t is extended beyond 0.5s then a larger number of terms will have to be calculated to obtain converged results.

If we denote $\bar{r}_C$ as the chemical randomness parameter, which takes backward steps into account, we found that,

$$\bar{r}_C = \frac{(2P_+ - 1)r_M - 4P_+(1 - P_+)}{(2P_+ - 1)^2}. \tag{S33}$$

Thus, $\bar{r}_C$ can be calculated from mechanical randomness parameter by taking into account backward steps. The derivation of this equation can be found in the literature in a different context [7, 8]. We show that in the next section this relation can be derived by including backward steps in the work by Schnitzer and Block [6]. In order to calculate $\bar{r}_C$ we need to compute the probability of forward step, P_+. We assume $P_+ + P_- = 1$ for all times until the motor detaches.

Thus, in our model,

$$P_+ = \frac{\int_0^\infty f_+(\tau_+)d\tau_+}{\int_0^\infty f_+(\tau_+)d\tau_+ + \int_0^\infty f_-(\tau_-)d\tau_-} = \frac{k^+}{k^+ + k^-}. \tag{S34}$$

Using r_M and P_+, we are able to calculate $\bar{r}_C$.

C. Derivation of mechanical randomness parameter with backward steps

We derive Eq.(S33) by incorporating backward steps into the work by Schnitzer and Block [6]. We denote the dwell time distribution corresponding to forward and backward steps as $f_+(t)$ and $f_-(t)$, respectively. Let $g(m, l, t)$ be the probability distribution of taking m forward steps and l backward steps before time t. The Laplace transform of $g(m, l, t)$, is written as follows,

$$\tilde{g}(m, l, s) = \frac{(m+l)!}{m!l!}(\tilde{f}_+(s))^m(\tilde{f}_-(s))^l \frac{1 - \tilde{f}_+(s) - \tilde{f}_-(s)}{s}. \tag{S35}$$

The last term in (S35) is the Laplace transform of $1 - \int_0^t (f_+(t) + f_-(t))dt$, means neither forward nor backward step occurs in the last time step. For $n \equiv m - l \geq 0$,

$$\begin{aligned}\tilde{g}_+(n, s) =& \sum_{m,l} \delta_{n,m-l}\tilde{g}(m, l, s) \\ =& [\tilde{f}_+(s)]^n \ {}_2\mathrm{F}_1\left(\frac{1+n}{2}, \frac{2+n}{2}, 1+n; 4\tilde{f}_+(s)\tilde{f}_-(s)\right)\frac{1 - \tilde{f}_+(s) - \tilde{f}_-(s)}{s}.\end{aligned} \tag{S36}$$

Using the special case of Hypergeometric function (page 556 of [1]) we obtain,

$$\tilde{g}_+(n,s) = \left[\frac{2\tilde{f}_+(s)}{1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}\right]^n \frac{1}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}} \frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}. \tag{S37}$$

For $n \leq 0$, a similar procedure leads to,

$$\tilde{g}_-(n,s) = \left[\frac{2\tilde{f}_-(s)}{1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}\right]^{-n} \frac{1}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}} \frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}. \tag{S38}$$

Thus,

$$\tilde{g}_\pm(n,s) = \left(\frac{2\tilde{f}_\pm(s)}{1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}\right)^{|n|} \frac{1}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}} \frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}. \tag{S39}$$

The first and second moment of n are defined as,

$$\begin{aligned}
\langle n(s)\rangle &= \sum_{n=0}^{\infty} n\tilde{g}_+(n,s) + \sum_{n=-\infty}^{0} n\tilde{g}_-(n,s) \\
&\equiv \langle n(s)\rangle_+ + \langle n(s)\rangle_- \\
\langle n^2(s)\rangle &= \sum_{n=0}^{\infty} n^2\tilde{g}_+(n,s) + \sum_{n=-\infty}^{0} n^2\tilde{g}_-(n,s) \\
&\equiv \langle n^2(s)\rangle_+ + \langle n^2(s)\rangle_-.
\end{aligned} \tag{S40}$$

It is convenient to use the generating function of $\tilde{g}_\pm(n,s)$ to calculate the moments, and it is a simple geometric sum given by,

$$\begin{aligned}
Z_+(x,s) &= \sum_{n=0}^{\infty} \tilde{g}_+(n,s)x^n \\
&= \frac{1}{1-\frac{2\tilde{f}_+(s)x}{1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}} \frac{1}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}} \frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}.
\end{aligned} \tag{S41}$$

The average number of forward step is calculated using the generating function as,

$$\begin{aligned}\langle n(s)\rangle_+ =&\frac{\partial Z_+}{\partial x}\Big|_{x=1}\\ =&\frac{2\tilde{f}_+(s)\Big[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big[1-2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]^2}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}.\end{aligned} \tag{S42}$$

The second derivative of the generating function leads to,

$$\begin{aligned}\langle n^2(s)\rangle_+ - \langle n(s)\rangle_+ =&\frac{\partial^2 Z_+}{\partial x^2}\Big|_{x=1}\\ =&\frac{8[\tilde{f}_+(s)]^2\Big[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big[1-2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]^3}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}.\end{aligned} \tag{S43}$$

Thus,

$$\langle n^2(s)\rangle_+ =\frac{2\tilde{f}_+\Big[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]\Big[1+2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big[1-2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]^3}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}. \tag{S44}$$

In a similar manner,

$$\langle n(s)\rangle_- = -\frac{2\tilde{f}_-(s)\Big[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big[1-2\tilde{f}_-(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big]^2}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}, \tag{S45}$$

$$\langle n^2(s)\rangle_- =\frac{2\tilde{f}_-\Big[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]\Big[1+2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\Big[1-2\tilde{f}_-(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\ \Big]^3}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}. \tag{S46}$$

By substituting the above expressions in Eq.(S40) we obtain,

$$\langle n(s)\rangle = \frac{2\left[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}\left[\frac{\tilde{f}_+(s)}{\left[1-2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]^2} - \frac{\tilde{f}_-(s)}{\left[1-2\tilde{f}_-(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]^2}\right], \tag{S47}$$

$$\langle n^2(s)\rangle = \frac{2\left[1+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]}{\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}}\frac{1-\tilde{f}_+(s)-\tilde{f}_-(s)}{s}\left[\frac{\tilde{f}_+(s)\left[1+2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]}{\left[1-2\tilde{f}_+(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]^3} + \frac{\tilde{f}_-(s)\left[1+2\tilde{f}_-(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]}{\left[1-2\tilde{f}_-(s)+\sqrt{1-4\tilde{f}_+(s)\tilde{f}_-(s)}\right]^3}\right]. \tag{S48}$$

We express $\tilde{f}_\pm(s)$ using Taylor expansion,

$$\begin{aligned}\tilde{f}_+(s) =& P_+\sum_{k=0}^{\infty}\frac{\langle\tau_+^k\rangle(-s)^k}{k!}\\ \tilde{f}_-(s) =& P_-\sum_{k=0}^{\infty}\frac{\langle\tau_-^k\rangle(-s)^k}{k!}.\end{aligned} \tag{S49}$$

The moments of $\tilde{f}_\pm(t)$ are given by,

$$\begin{aligned}\langle\tau_+^k\rangle =& \frac{\int_0^\infty \tau_+^k f_+(\tau_+)d\tau_+}{\int_0^\infty f_+(\tau_+)d\tau_+} = \frac{\int_0^\infty \tau_+^k f_+(\tau_+)d\tau_+}{P_+} = \frac{(-1)^k\frac{d^k\tilde{f}_+(s)}{ds^k}}{P_+},\\ \langle\tau_-^k\rangle =& \frac{\int_0^\infty \tau_-^k f_-(\tau_-)d\tau_-}{\int_0^\infty f_-(\tau_-)d\tau_-} = \frac{\int_0^\infty \tau_-^k f_-(\tau_-)d\tau_-}{P_-} = \frac{(-1)^k\frac{d^k\tilde{f}_-(s)}{ds^k}}{P_-}.\end{aligned} \tag{S50}$$

From here we assume the relation $P_+ + P_- = 1$. Substituting (S49) into (S47) and (S48) with the condition either $\frac{1}{2} < P_+ \leq 1$ or $0 \leq P_+ < \frac{1}{2}$ both yield,

$$\begin{aligned}\langle n(s)\rangle =&\frac{2P_+ - 1}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)s^2}\\&+\frac{-2P_+^2\langle\tau_+\rangle^2 + 2(1-P_+)^2\langle\tau_-\rangle^2 + P_+(2P_+-1)\langle\tau_+^2\rangle + (2P_+-1)(1-P_+)\langle\tau_-^2\rangle}{2\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2 s} + O(1),\end{aligned} \tag{S51}$$

and

$$\begin{aligned}\langle n^2(s)\rangle =&\frac{2(2P_+-1)^2}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2 s^3}+\\&\frac{-P_+^2(8P_+-5)\langle\tau_+\rangle^2 + (1-P_+)^2(8P_+-3)\langle\tau_-\rangle^2 + 2(2P_+-1)^2\big((1-P_+)\langle\tau_-^2\rangle + P_+\langle\tau_+^2\rangle\big) + 2(1-P_+)P_+\langle\tau_+\rangle\langle\tau_-\rangle}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^3 s^2}\\&+ O(s^{-1}).\end{aligned} \tag{S52}$$

After inverse Laplace transform,

$$\begin{aligned}\langle n(t)\rangle =&\frac{2P_+ - 1}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)}t\\&+\frac{-2P_+^2\langle\tau_+\rangle^2 + 2(1-P_+)^2\langle\tau_-\rangle^2 + P_+(2P_+-1)\langle\tau_+^2\rangle + (2P_+-1)(1-P_+)\langle\tau_-^2\rangle}{2\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2} + O(t^{-1}),\end{aligned} \tag{S53}$$

and

$$\begin{aligned}\langle n^2(t)\rangle - \langle n(t)\rangle^2 =&\frac{P_+(2P_+-1)^2\langle\tau_+^2\rangle - P_+^2(4P_+-3)\langle\tau_+\rangle^2}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^3}t\\&+\frac{(1-P_+)(2P_+-1)^2\langle\tau_-^2\rangle - (1-P_+)^2(1-4P_+)\langle\tau_-\rangle^2}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^3}t\\&+\frac{2(1-P_+)P_+\langle\tau_+\rangle\langle\tau_-\rangle}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^3}t + O(1).\end{aligned} \tag{S54}$$

Now we compute the randomness parameter as

$$
\begin{aligned}
r_M &= \lim_{t\to\infty} \frac{\langle n^2(t)\rangle - \langle n(t)\rangle^2}{\langle n(t)\rangle} \\
&= \frac{P_+(2P_+-1)^2\langle \tau_+^2\rangle - P_+^2(4P_+-3)\langle \tau_+\rangle^2}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2(2P_+-1)} \\
&\quad + \frac{(1-P_+)(2P_+-1)^2\langle \tau_-^2\rangle - (1-P_+)^2(1-4P_+)\langle \tau_-\rangle^2}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2(2P_+-1)} \\
&\quad + \frac{2(1-P_+)P_+\langle\tau_+\rangle\langle\tau_-\rangle}{\big(P_+\langle\tau_+\rangle + (1-P_+)\langle\tau_-\rangle\big)^2(2P_+-1)}.
\end{aligned} \tag{S55}
$$

For the special case, namely $\langle\tau_+\rangle = \langle\tau_-\rangle$ and $\langle\tau_+^2\rangle = \langle\tau_-^2\rangle$, r_M in the above equation reduces to the following expression,

$$
r_M = \frac{(2P_+-1)^2\langle\tau^2\rangle - (8P_+^2 - 8P_+ + 1)\langle\tau\rangle^2}{(-1+2P_+)\langle\tau\rangle^2}. \tag{S56}
$$

Manipulation of Eq.($S56$) leads to,

$$
r_C = \frac{(2P_+-1)r_M - 4P_+(1-P_+)}{(2P_+-1)^2} \equiv \bar{r}_C, \tag{S57}
$$

where $r_C = \frac{\langle\tau^2\rangle - \langle\tau\rangle^2}{\langle\tau\rangle^2}$. Note that $r_C = r_M$ if P_+ is unity, which holds in the absence of backward steps.

IV. TWO MODELS FOR HOW KINESIN WAITS FOR ATP

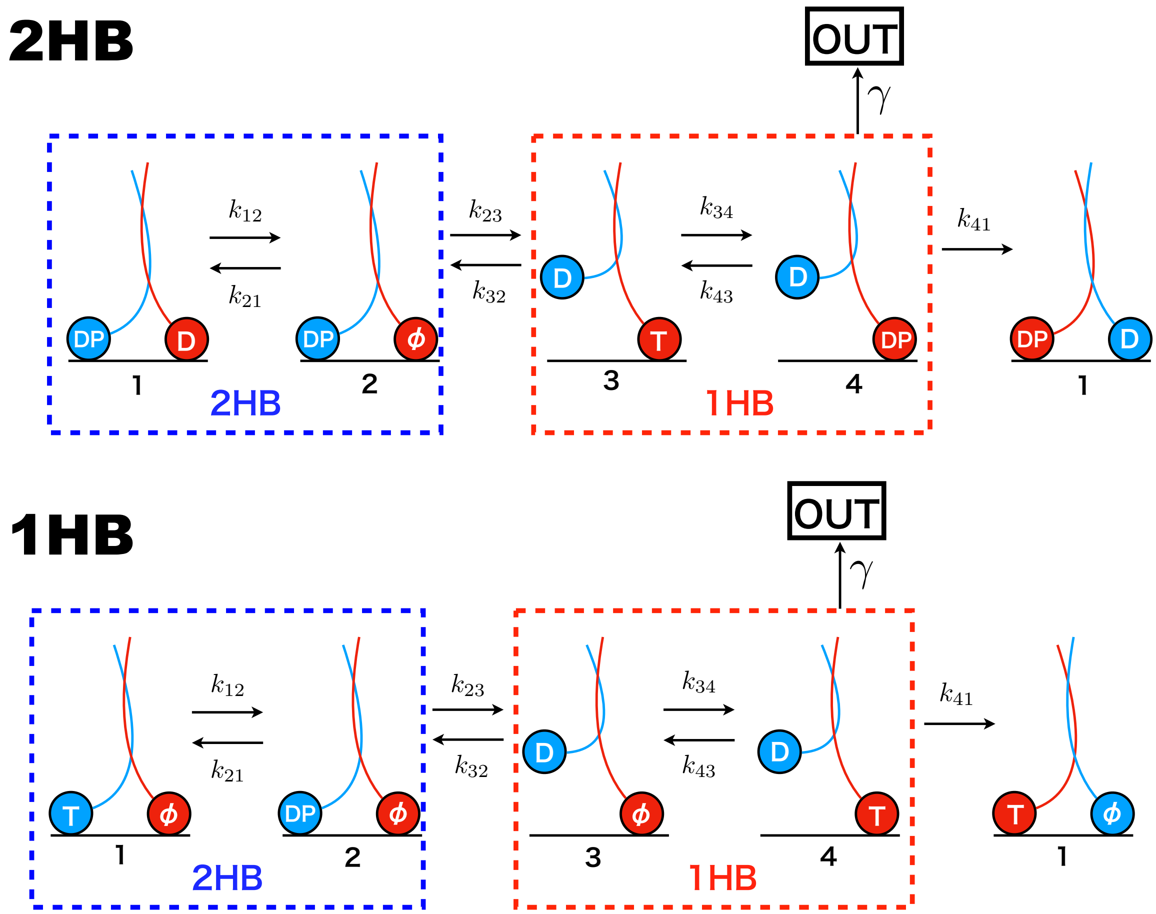

FIG. S2. A sketch showing all the relevant rates involved in the stepping of kinesin. The upper (lower) panel shows the 2HB (1HB) waiting state. In the 2HB model , ATP binds to the LH when both heads are bound to the microtuble (MT). In the 1HB model ATP binding occurs only after the trailing head detaches from the MT. At extremely low ATP concentration the 1HB model is more likely.

As explained in the main text, the two models (Fig.S3) have been proposed for the waiting states of kinesin for ATP binding. In the 2HB model, ATP binds to the leading head when both the heads are bound to the MT (upper panel in Fig.S2). In contrast, Isojima *et al.* have suggested, based on dark field microscopy, that ATP binds only after the trailing head detaches leading to the so-called vulnerable state (Fig.S3 bottom panel). In order to simplify the kinetic scheme, we merge the 4 states in Fig.S2 into two states shown in Fig.S3 in order

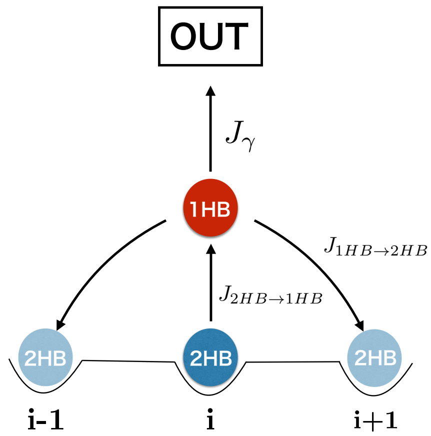

FIG. S3. Simplified two states model.

to calculate the net flux of transition from 2HB state to 1HB state ($J_{2HB\to 1HB}$), forward step ($J_{1HB\to 2HB}$), and detachment (J_γ). The simplification allows us to obtain closed form expressions for $J_{2HB\to 1HB}$, $J_{1HB\to 2HB}$, and J_γ.

In the following calculations, we employ method pioneered by Hill [9, 10]. We may design state 3 to be the absorbing state for the transition from 2HB to 1HB (state 2 to state 3), state 1 to be the absorbing state for the transition from 1HB to 2HB (state 4 to state 1). In addition, detachment is also an absorbing state when kinesin is in the 1HB state. By obtaining the stationary solution of the following sets of master equations, with the conditions $P_1 + P_2 = 1$ and $P_3 + P_4 = 1$,

$$\begin{aligned} \frac{dP_1}{dt} &= -k_{12}P_1 + (k_{23} + k_{21})P_2, \\ \frac{dP_2}{dt} &= k_{12}P_1 - (k_{21} + k_{23})P_2, \end{aligned} \tag{S58}$$

$$\begin{aligned} \frac{dP_3}{dt} &= -k_{34}P_3 + (k_{43} + k_{41} + \gamma)P_2, \\ \frac{dP_4}{dt} &= k_{34}P_3 - (k_{41} + k_{43} + \gamma)P_2, \end{aligned} \tag{S59}$$

we obtain,

$$\begin{aligned} J_{2HB\to 1HB} &= \frac{k_{12}k_{23}}{k_{12}+k_{21}+k_{23}}, \\ J_{1HB\to 2HB} &= \frac{k_{34}k_{41}}{\gamma+k_{34}+k_{41}+k_{43}}, \\ J_{\gamma} &= \frac{\gamma k_{34}}{\gamma+k_{34}+k_{41}+k_{43}}, \end{aligned} \tag{S60}$$

ATP binds with the rate k_{23} in the 2HB waiting state (upper panel in Fig.S2), which we parametrize as $k_{23} = [\mathrm{T}]k^0_{23}$, where [T] is the ATP concentration. On the other hand, ATP binding occurs with rate k_{34} in the 1HB model with detached TH. Thus, we include ATP dependence in $k_{34} = [\mathrm{T}]k^0_{34}$. With these assumptions, the expressions for the fluxes are given by,

$$\begin{aligned} J_{2HB\to 1HB} &= \frac{k_{12}[\mathrm{T}]k^0_{23}}{k_{12}+k_{21}+[\mathrm{T}]k^0_{23}}, \\ J_{1HB\to 2HB} &= \frac{k_{34}k_{41}}{\gamma+k_{34}+k_{41}+k_{43}}, \\ J_{\gamma} &= \frac{\gamma k_{34}}{\gamma+k_{34}+k_{41}+k_{43}}, \end{aligned} \tag{S61}$$

in the 2HB mdoel. The analogous expressions in the 1HB model are,

$$\begin{aligned} J_{2HB\to 1HB} &= \frac{k_{12}k_{23}}{k_{12}+k_{21}+k_{23}}, \\ J_{1HB\to 2HB} &= \frac{[\mathrm{T}]k^0_{34}k_{41}}{\gamma+[\mathrm{T}]k^0_{34}+k_{41}+k_{43}}, \\ J_{\gamma} &= \frac{\gamma[\mathrm{T}]k^0_{34}}{\gamma+[\mathrm{T}]k^0_{34}+k_{41}+k_{43}}, \end{aligned} \tag{S62}$$

Thus, the Michaelis-menten (MM) kinetics naturally arises from this coarse grained procedure in $J_{2HB\to 1HB}$ (Eq.S61) and $J_{1HB\to 2HB}$ (Eq.S62). Since the MM constant in $J_{1HB\to 2HB}$ and J_γ for the 1HB waiting model are identical, we used the functional form $k^+ = \frac{k_0^+[\mathrm{T}]}{K_T+[\mathrm{T}]}\mathrm{e}^{-\beta F d^+}$, $k^- = \frac{k_0^-[\mathrm{T}]}{K_T+[\mathrm{T}]}\mathrm{e}^{\beta F d^-}$, and $\gamma = \frac{\gamma_0[\mathrm{T}]}{K_T+[\mathrm{T}]}\mathrm{e}^{F/F_d}$. Using identical MM constant in k^+, k^-, and γ in 1HB waiting model leads to ATP independent ratio of probability of forward step, backward step, and detachment. This is realized in 2the HB waiting model as well, and is in accord with experimental observation [11, 12].

k_0	$244.0(s^{-1})$	d^+	2.2 (nm)
k_0^+	$303.1(s^{-1})$	d^-	0.7(nm)
k_0^-	$1.3(s^{-1})$	F_d	3.0(pN)
γ_0	$2.4(s^{-1})$	K_T	16.0(μM)

TABLE S1. Extracted parameters for the variant of 1HB model

V. VARIANT OF 1HB MODEL: k^- IS INDEPENDENT OF [T]

It appears logical that the backward step should be the reverse of the forward step, which implies that it too should occur by a hand-over-hand mechanism with the rate being dependent on ATP. For reasons discussed in the main text, depending on the pathway that kinesin takes to go take a backward step, it is possible that rate of backward step k^- does not depend on ATP binding. Therefore, we created a variant of the 1HB model [Fig.1(c) in the main text] in which k^- is independent of [T]. The load dependence is identical to the original 1HB model, namely $k^- = k_0^- \mathrm{e}^{\beta F_d^-}$. We followed the same procedure described in the main text to obtain the parameters for the variant of 1HB model, which are listed in Table.S1. Interestingly, the values of the many parameters extracted using the 1HB and variant 1HB models are not that dissimilar (compare Table.S1 and Table 2 in the main text). We show in Fig.S4 that our main prediction about the qualitative behavior of randomness parameters are robust: the randomness parameters for the variant of 1HB waiting model also show monotonic decrease as [T] is increased. Thus, regardless of the mechanism of the backward step we conclude that measurements of the randomness parameter as a function of load and ATP concentration using currently available high temporal resolution experiments should resolve the nature of waiting states for ATP.

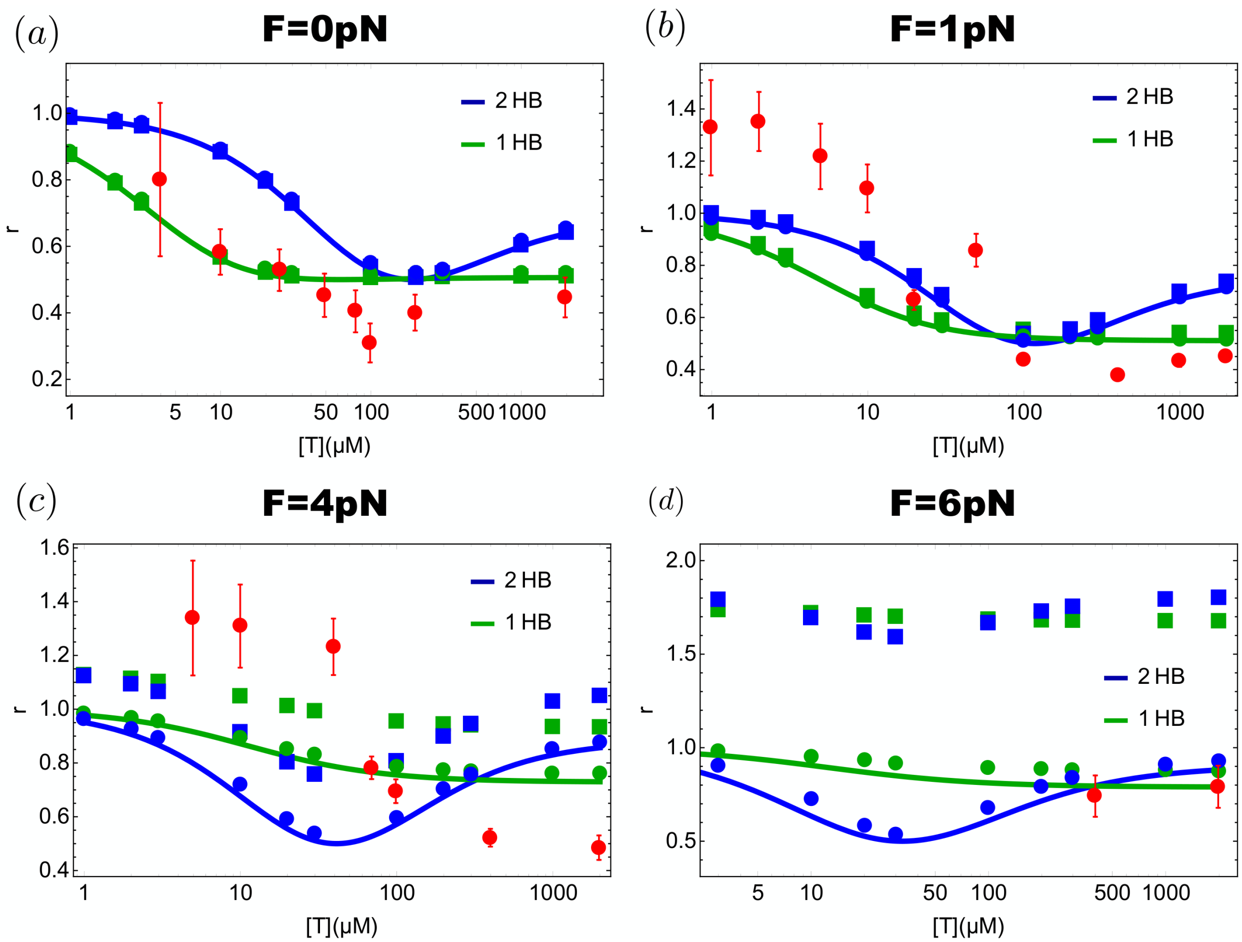

FIG. S4. Theoretical predictions for the ATP concentration dependence of the three randomness parameters, r_M, r_C, and $\bar{r}_C$ at different external loads for the 2HB model (blue) and the variant of 1HB model (green). Filled circles, filled squares and lines denote r_M, $\bar{r}_C$, and r_C, respectively. Red circles with error bar in (a) are the experimentally measured randomness parameter at $F = 0$ in [2]. Red circles with error bar in (b)-(d) are the randomness parameters measured in [3]; (b) for 1.05 pN, (c) for 3.59 pN, and (d) for 5.69 pN. As explained in the discussion section in the main text, randomness parameters in our schemes are always equal or grater than 0.5. The results for the 1HB model are obtained by assuming that the rate for the backward step is a constant independent of the ATP concentration.

VI. FITTING THEORY TO EXPERIMENTAL DATA

In order to obtain the parameters for the model, we analyzed the distribution of run length and velocity at $F = 0$ using the data reported in Ref.[13]. For Kin1, the measured mean velocity at 0 load is 1089 nm/s, which implies $J^+ - J^- = 132.8$ step/s. Since J^+/J^- is not given in Ref.[13], we used the value of J^+/J^- obtained in Ref.[11], $J^+/J^- = 221$. We set $F_d = 3$pN [14, 15] and used the constraint $|d^+| + |d^-| = 2.9$nm [11]. By fitting to the

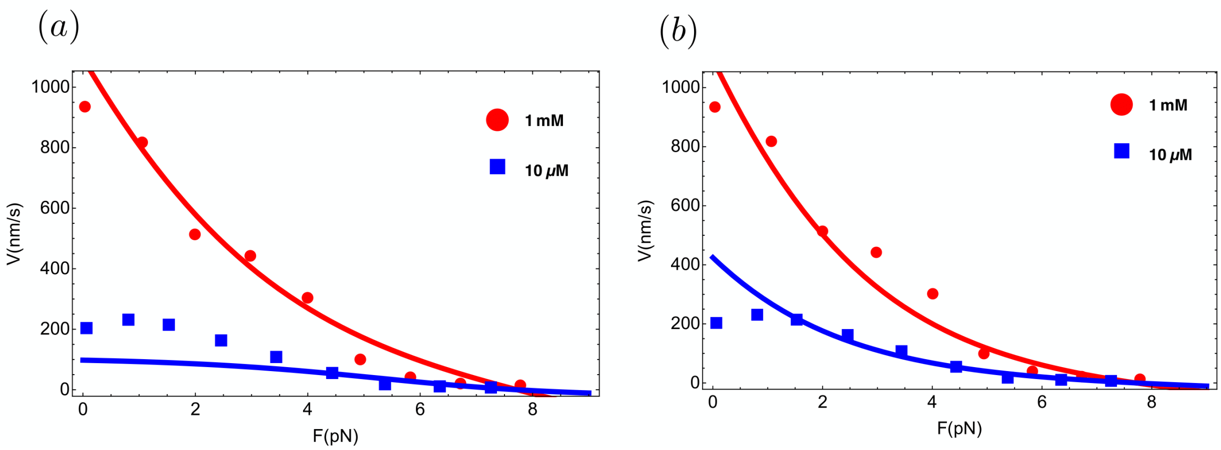

FIG. S5. Fits of the of average velocity as a function of load to the experiment [11]. (a) 2HB waiting model [Fig.1(c) in the main text]. (b) 1HB waiting model [Fig.1(d) in the main text]. The agreement between theory and experiment is good.

run length distribution using our theory [Eq.(S3)] along with the 4 constraints described above are used to determine the model parameters at zero load, k_0, k_0^+, k_0^-, γ_0, and K_T. Subsequently, we used the data for average velocity vs load at different ATP concentrations in Ref.[11] to obtain d^+ and d^-. The best fit parameters are listed in Table.1 and Table.2 in the main text for the 2HB waiting and 1HB waiting model, respectively.

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