Supplementary Information:
Kinetic analysis methods applied to single motor protein trajectories

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Note added after first publication: *This supplementary information file replaces the version published on the 2nd of July 2018, which contained mistakes in different equations of sec.1.*

Contents

1 Derivations of equations

Below we provide derivations of various equations from the manuscript.

1.1 The variance of bead position: Equation (7)

The force balance Equation (2) from the manuscript can be rewritten as,

$$\mathrm{d}x_\mathrm{b}(t) = \frac{k_l}{\gamma}(x_\mathrm{m}(t) - x_\mathrm{b}(t))\,\mathrm{d}t + \frac{\xi(t)}{\gamma}\,\mathrm{d}t, \tag{S1}$$

where the thermal noise satisfies

$$\langle \xi(t) \rangle = 0, \quad \langle \xi(t)\xi(t') \rangle = 2k_\mathrm{B}T\gamma\,\delta(t-t'), \quad t, t' > 0.$$

Equation (S1) can be integrated from time 0 to time t to give,

$$x_\mathrm{b}(t) = x_\mathrm{b}(0)e^{-k_l t/\gamma} + \int_0^t \mathrm{d}t'\, e^{-k_l(t-t')/\gamma}\left(\frac{k_l}{\gamma}x_\mathrm{m}(t') + \frac{\xi(t')}{\gamma}\right). \tag{S2}$$

We are interested in calculating how the steady-state variance of the bead position changes over the time interval Δt. As we are considering the steady state, the average bead velocity is constant and equals the average motor velocity $v = \langle v_\mathrm{b} \rangle = \langle v_\mathrm{m} \rangle$, and we can write

$$\begin{aligned}
\mathrm{var}(x_\mathrm{b}(\Delta t)) &= \lim_{T\to\infty}\frac{1}{T}\int_0^T \mathrm{d}t' \langle (x_\mathrm{b}(t'+\Delta t) - x_\mathrm{b}(t') - v\Delta t)^2 \rangle \\
&= [\Delta x_\mathrm{b}(t) = x_\mathrm{b}(t) - vt] \\
&= \lim_{T\to\infty}\frac{1}{T}\int_0^T \mathrm{d}t' \langle [\Delta x_\mathrm{b}(t'+\Delta t) - \Delta x_\mathrm{b}(t')]^2 \rangle \\
&= \lim_{T\to\infty}\frac{1}{T}\int_0^T \mathrm{d}t' \left[\langle \Delta x_\mathrm{b}^2(t'+\Delta t)\rangle + \langle \Delta x_\mathrm{b}^2(t')\rangle - 2\langle \Delta x_\mathrm{b}(t'+\Delta t)\Delta x_\mathrm{b}(t')\rangle\right].
\end{aligned}$$

As the integrand does not vanish for large times t', the integral will be dominated by contributions at large times as $T \to \infty$, and we can write

$$\mathrm{var}(x_\mathrm{b}(\Delta t)) = \lim_{t'\to\infty} \langle \Delta x_\mathrm{b}^2(t'+\Delta t)\rangle + \langle \Delta x_\mathrm{b}^2(t')\rangle - 2\langle \Delta x_\mathrm{b}(t'+\Delta t)\Delta x_\mathrm{b}(t')\rangle.$$

To calculate this, it will be useful to know the cross-correlation,

$$\langle \Delta x_\mathrm{b}(t)\Delta x_\mathrm{b}(t')\rangle = \langle (x_\mathrm{b}(t) - vt)(x_\mathrm{b}(t') - vt')\rangle = \langle x_\mathrm{b}(t)x_\mathrm{b}(t')\rangle - v^2tt',$$

where

$$\begin{aligned}\langle x_{\rm b}(t)x_{\rm b}(t')\rangle &= \langle x_{\rm b}^2(0)\rangle e^{-k_l(t+t')/\gamma}+\\ &+\int\limits_0^t {\rm d}t_1\int\limits_0^{t'}{\rm d}t_2\, e^{-k_l(t'-t_2)/\gamma-k_l(t-t_1)/\gamma}\Big\langle\Big(\frac{k_l}{\gamma}x_{\rm m}(t_1)+\frac{\xi(t_1)}{\gamma}\Big)\Big(\frac{k_l}{\gamma}x_{\rm m}(t_2)+\frac{\xi(t_2)}{\gamma}\Big)\Big\rangle,\end{aligned}$$

and we have used the fact that neither motor position nor noise depend on the original position of the bead, $x_{\rm b}(0)$, and we have defined the coordinates such that $x_{\rm m}(0)=0$, giving $\langle x_{\rm b}(0)\rangle=0$. To calculate the initial strength of the bead fluctuations we consider the evolution of the bead before the motor starts,

$$\begin{gathered}{\rm d}x_{\rm b}(t) = -\frac{k_l}{\gamma}x_{\rm b}(t)\,{\rm d}t+\frac{\xi(t)}{\gamma}\,{\rm d}t,\\ \langle\xi(t)\rangle=0,\quad \langle\xi(t)\xi(t')\rangle = 2k_{\rm B}T\gamma\,\delta(t-t'),\quad t,t'<0.\end{gathered}\tag{S3}$$

Using this we have

$$\langle x_{\rm b}^2(0)\rangle = \int\limits_{-\infty}^0{\rm d}t'\int\limits_{-\infty}^0{\rm d}t''\,e^{-k_l(-t'-t'')/\gamma}\frac{\langle\xi(t')\xi(t'')\rangle}{\gamma^2}=\frac{2k_{\rm B}T}{\gamma}\int\limits_0^\infty{\rm d}t'\,e^{-2k_lt'/\gamma}=\frac{k_{\rm B}T}{k_l}.$$

If we assume the thermal noise to be independent of motor position, we have $\langle\xi(t)x_{\rm m}(t')\rangle = \langle\xi(t)\rangle\langle x_{\rm m}(t')\rangle=0$, and can write

$$\Big\langle\Big(\frac{k_l}{\gamma}x_{\rm m}(t_1)+\frac{\xi(t_1)}{\gamma}\Big)\Big(\frac{k_l}{\gamma}x_{\rm m}(t_2)+\frac{\xi(t_2)}{\gamma}\Big)\Big\rangle=\Big(\frac{k_l}{\gamma}\Big)^2\langle x_{\rm m}(t_1)x_{\rm m}(t_2)\rangle+\frac{2k_{\rm B}T}{\gamma}\,\delta(t_1-t_2)\,.$$

As the motor stepping is uncorrelated, and assuming $t_1>t_2$, we move to the limit of discrete stepping, and have

$$\begin{aligned}\langle x_{\rm m}(t_1)x_{\rm m}(t_2)\rangle &= \int\limits_0^{t_1}{\rm d}t_1'\int\limits_0^{t_2}{\rm d}t_2'\langle v_{\rm m}(t_1')v_{\rm m}(t_2')\rangle = \lim_{\Delta t\to0}\sum_{t_1'}\Delta t\sum_{t_2'}\Delta t\langle v_{\rm m}(t_1')v_{\rm m}(t_2')\rangle\\ &= \lim_{\Delta t\to0}\left(\sum_{t_1'\neq t_2}\Delta t^2\langle v_{\rm m}(t_1')\rangle\langle v_{\rm m}(t_2')\rangle+\sum_{t_2'}\Delta t^2\langle v_{\rm m}^2(t_2')\rangle\right).\end{aligned}$$

As the terms in the first sum are regular as $t_1\to t_2$, we can again take the continuum limit for this term and arrive at

$$\begin{aligned}\langle x_{\rm m}(t_1)x_{\rm m}(t_2)\rangle &= \int\limits_0^{t_1}{\rm d}t_1'\int\limits_0^{t_2}{\rm d}t_2'\langle v_{\rm m}(t_1')\rangle\langle v_{\rm m}(t_2')\rangle+\lim_{\Delta t\to0}\sum_{t_2'}\Delta t^2\langle v_{\rm m}^2(t_2')\rangle\\ &= v_{\rm m}^2t_1t_2+\lim_{\Delta t\to0}\sum_{t_2'}\Delta t^2\langle v_{\rm m}^2(t_2')\rangle.\end{aligned}$$

in the steady state. The instantaneous motor velocity is given by

$$v_{\rm m}(t) = \sum_i a\delta(t - t_i), \tag{S4}$$

where a is the size of the mechanical step of the motor, t_i is the time at which the i^{th} step is taken, distributed over $[0, \infty)$ with density v/a (the number of steps per time). We then have (we use $v = v_m$),

$$\lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 \langle v^2(t_2')\rangle = \lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 \Big\langle \sum_{i,j} a^2\, \delta(t_2' - t_i)\, \delta(t_2' - t_j) \Big\rangle.$$

The sum within the average has non-zero values only when $i = j$, giving,

$$\begin{aligned}
\lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 \langle v^2(t_2')\rangle &= \lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 \Big\langle \sum_{i} a^2\, \delta^2(t_2' - t_i) \Big\rangle \\
&= \lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 \int_0^\infty {\rm d}t_i\, \frac{v}{a} a^2 \delta^2(t_2 - t_i) = \lim_{\Delta t\to 0} \sum_{t_2'} \Delta t^2 va\, \delta(0) \\
&= \lim_{\Delta t\to 0} \sum_{t_2'} \Delta t va = vat_2
\end{aligned}$$

In the above, we have used that when discrete, $\delta(0) = \lim_{\Delta t\to 0} 1/\Delta t$. Thus, the correlation of motor position is,

$$\langle x_{\rm m}(t_1) x_{\rm m}(t_2)\rangle = v^2 t_1 t_2 + va \min(t_1, t_2).$$

Returning to the correlation of bead position, we have,

$$\langle x_{\rm b}(t) x_{\rm b}(t'))\rangle = \frac{k_{\rm B}T}{k_l} + A + B + C,$$

with,

$$\begin{aligned}
A &= \left(\frac{k_l v}{\gamma}\right)^2 \int_0^t {\rm d}t_1\, e^{-k_l(t - t_1/\gamma)} t_1 \int_0^{t'} {\rm d}t_2\, e^{-k_l(t' - t_2/\gamma)} t_2 \\
B &= \left(\frac{k_l}{\gamma}\right)^2 va \int_0^t {\rm d}t_1 \int_0^{t'} {\rm d}t_2\, e^{-k_l(t' - t_2)/\gamma - k_l(t' - t_1)/\gamma} \min(t_1, t_2) \\
C &= \int_0^t {\rm d}t_1 \int_0^{t'} {\rm d}t_2\, e^{-k_l(t' - t_2)/\gamma - k_l(t' - t_1)/\gamma} \frac{2k_{\rm B}T}{\gamma}\, \delta(t_1 - t_2)\,.
\end{aligned}$$

Considering the above term by term,

$$A = v^2 \Big[t - \frac{\gamma}{k_l}(1 - e^{-k_l t/\gamma})\Big] \Big[t' - \frac{\gamma}{k_l}(1 - e^{-k_l t'/\gamma})\Big].$$

Assuming $t > t'$, we can write,

$$\begin{aligned} B &= \left(\frac{k_l}{\gamma}\right)^2 va\Big[\int_0^{t'} \mathrm{d}t_2 \int_0^{t_2} \mathrm{d}t_1 e^{-k_l(t'-t_2)/\gamma - k_l(t-t_1)/\gamma} t_1 + \\ &+ \int_0^{t'} \mathrm{d}t_2 \int_{t_2}^{t} \mathrm{d}t_1 e^{-k_l(t'-t_2)/\gamma - k_l(t-t_1)/\gamma} vat_2\Big] \\ &= av\Big(t' - \frac{\gamma}{2k_l}(1-e^{-k_l t'/\gamma})^2 - \frac{\gamma}{2k_l}(1-e^{-k_l t'/\gamma})(1+e^{-k_l(t-t')/\gamma})\Big). \end{aligned}$$

Again assuming $t > t'$, the integral over t_1 in C contributes nothing for $t_1 > t'$, as $t_2 < t'$ giving,

$$\begin{aligned} C &= \int_0^{t'} \mathrm{d}t_1 \int_0^{t'} \mathrm{d}t_2\, e^{-k_l(t'-t_2)/\gamma - k_l(t-t_1)/\gamma} \frac{2k_\mathrm{B}T}{\gamma}\delta(t_1 - t_2) \\ &= e^{-k_l(t'+t)/\gamma}\frac{k_\mathrm{B}T}{k_l}(e^{2k_l t'/\gamma} - 1) = \frac{k_\mathrm{B}T}{k_l}(e^{-k_l(t-t')/\gamma} - e^{-k_l(t'+t)/\gamma}) \end{aligned}$$

Taken together, we have,

$$\begin{aligned} \langle \Delta x_\mathrm{b}(t+\Delta t)\Delta x_\mathrm{b}(t)\rangle &= \langle x_\mathrm{b}(t+\Delta t)x_\mathrm{b}(t) - v^2 t(t+\Delta t) \\ &= \frac{k_\mathrm{B}T}{k} - v^2(t+\Delta t)\frac{\gamma}{k_l}(1-e^{-k_l t/\gamma}) - v^2 t\frac{\gamma}{k_l}(1-e^{-k_l(t+\Delta t)/\gamma}) \\ &+ v^2\Big[\frac{\gamma}{k_l}(1-e^{-k_l(t+\Delta t)/\gamma})\Big]\Big[\frac{\gamma}{k_l}(1-e^{-k_l/\gamma})\Big] \\ &- av\Big(t - \frac{\gamma}{2k_l}(1-e^{-k_l t/\gamma})^2 - \frac{\gamma}{2k_l}(1-e^{-k_l t/\gamma})(1-e^{-k_l \Delta t/\gamma})\Big) \\ &+ \frac{k_\mathrm{B}T}{k_l}(e^{-k_l\Delta t/\gamma} - e^{-k_l(2t+\Delta t)/\gamma}). \end{aligned}$$

As we are interested in the steady state regime, we take the long-time limit $t \to \infty$ to arrive at,

$$\begin{aligned} \lim_{t\to\infty}\langle \Delta x_\mathrm{b}(t+\Delta t)\Delta x_\mathrm{b}(t)\rangle &= \lim_{t\to\infty}\Bigg[\frac{k_\mathrm{B}T}{k_l}(1+e^{-k_l\Delta t/\gamma}) - v^2(t+\Delta t)\frac{\gamma}{k_l} - v^2 t\frac{\gamma}{k_l} + \left(\frac{v\gamma}{k_l}\right)^2 \\ &\quad -av(t+\Delta t - \frac{\gamma}{2k_l}(2+e^{-k_l\Delta t/\gamma}))\Bigg], \end{aligned}$$

as well as,

$$\lim_{t\to\infty}\langle \Delta x_\mathrm{b}^2(t+\Delta t)\rangle = \lim_{t\to\infty}\left[\frac{2k_\mathrm{B}T}{k_l} - 2v^2\frac{\gamma}{k_l}(t+\Delta t) + \left(\frac{v\gamma}{k_l}\right)^2 - av(t+\Delta t - \frac{3\gamma}{2k_l})\right],$$

and,

$$\lim_{t\to\infty}\langle x_\mathrm{b}^2(t)\rangle = \lim_{t\to\infty}\left[\frac{2k_\mathrm{B}T}{k_l} - 2v^2\frac{\gamma}{k_l}t + \left(\frac{v\gamma}{k_l}\right)^2 - av(t - \frac{3\gamma}{2k_l})\right].$$

Finally, bringing this all together we have that in the steady state

$$\begin{aligned}
\mathrm{var}(x_\mathrm{b}(\Delta t)) &= \lim_{t\to\infty} \langle \Delta x_\mathrm{b}^2(t+\Delta t)\rangle + \langle x_\mathrm{b}^2(t)\rangle - 2\langle \Delta x_\mathrm{b}(t+\Delta t)\Delta x_\mathrm{b}(t)\rangle \\
&= \lim_{t\to\infty} \Bigg[\frac{2k_\mathrm{B}T}{k_l} - 2v^2\frac{\gamma}{k_l}(t+\Delta t) + (\frac{v\gamma}{k_l})^2 - av(t+\Delta t - \frac{3\gamma}{2k_l}) + \frac{2k_\mathrm{B}T}{k_l} \\
&\quad -2v^2\frac{\gamma}{k_l}t + \left(\frac{v\gamma}{k_l}\right)^2 - av(t - \frac{3\gamma}{2k_l}) - \frac{2k_\mathrm{B}T}{k_l}(1+e^{-k_l\Delta t/\gamma}) \\
&\quad +2v^2\frac{\gamma}{k_l}(t+\Delta t) + 2v^2\frac{\gamma}{k_l}t - 2(\frac{v\gamma}{k_l})^2 \qquad \text{(S5)} \\
&\quad +2av(t+\Delta t - \frac{\gamma}{2k_l}(2+e^{-k_l\Delta t/\gamma}))\Bigg] \\
&= \frac{2k_\mathrm{B}T + av\gamma}{k_l}(1-e^{-k_l\Delta t/\gamma}) + av\Delta t \qquad \text{(S6)}
\end{aligned}$$

where we see that any dependence on t drops out, as expected in the steady state.

1.2 PSD of the speed of the bead: Equation (8)

The power-spectral density (PSD) of the function $f(t)$ is defined by,

$$\mathrm{PSD}_f(\omega) = \lim_{T\to\infty} \frac{1}{T}\langle |\mathcal{F}[f](\omega)|^2\rangle \qquad \text{(S7)}$$

where the Fourier transform of $f(t)$ is,

$$\mathcal{F}[f](\omega) = \int_{-T/2}^{T/2} \mathrm{d}t f(t) e^{-i\omega t}. \qquad \text{(S8)}$$

The equation of motion for the bead position x_b, given the position of the motor x_m and the thermal noise $\xi(t)$ is given by Equation (S1). The Fourier transform of Equation (S1) is,

$$i\omega\gamma\mathcal{F}[x_\mathrm{b}](\omega) = k\mathcal{F}[x_\mathrm{m}] - k\mathcal{F}[x_\mathrm{b}] + \mathcal{F}[\xi], \qquad \text{(S9)}$$

giving,

$$\mathcal{F}[x_\mathrm{b}](\omega) = \frac{k\mathcal{F}[x_\mathrm{m}] + \mathcal{F}[\xi]}{k + i\omega\gamma}.$$

The PSD of the velocity of the bead $v_\mathrm{b} = \mathrm{d}x_\mathrm{b}/\mathrm{d}t$ is then (where $v_\mathrm{m} = \mathrm{d}x_\mathrm{m}/\mathrm{d}t$ is the velocity of the motor),

$$\begin{aligned}
\mathrm{PSD}_{v_\mathrm{b}}(\omega) &= \lim_{T\to\infty}\frac{1}{T}\langle|\mathcal{F}[\frac{\mathrm{d}x_\mathrm{b}}{\mathrm{d}t}](\omega)|^2\rangle = \lim_{T\to\infty}\frac{1}{T}\langle|i\omega\mathcal{F}[x_\mathrm{b}]|^2\rangle && \text{(S10)}\\
&= \lim_{T\to\infty}\frac{1}{T}\langle\left|i\omega\frac{k\mathcal{F}[x_\mathrm{m}]+\mathcal{F}[\xi]}{k+i\omega\gamma}\right|^2\rangle = \lim_{T\to\infty}\frac{1}{T}\langle\left|\frac{k\mathcal{F}[v_\mathrm{m}]+i\omega\mathcal{F}[\xi]}{k+i\omega\gamma}\right|^2\rangle && \text{(S11)}\\
&= \lim_{T\to\infty}\frac{1}{T}\langle\left|\frac{k\mathcal{F}[v_\mathrm{m}]}{k+i\omega\gamma}+i\omega\frac{\mathcal{F}[\xi]}{k+i\omega\gamma}\right|^2\rangle && \text{(S12)}\\
&= \lim_{T\to\infty}\frac{1}{T}(\langle\left|\frac{k\mathcal{F}[v_\mathrm{m}]}{k+i\omega\gamma}\right|^2\rangle+\langle\left|i\omega\frac{\mathcal{F}[\xi]}{k+i\omega\gamma}\right|^2\rangle && \text{(S13)}\\
&= \frac{k^2}{k^2+\omega^2\gamma^2}\mathrm{PSD}_{v_\mathrm{m}}(\omega)+\frac{\omega^2}{k^2+\omega^2\gamma^2}\mathrm{PSD}_\xi(\omega). && \text{(S14)}
\end{aligned}$$

where we have used the fact that motor position and noise is uncorrelated in the second to last step.

1.3 PSD of motor velocity for more than one rate-limiting step: Equation (12)

The power spectrum of a stationary signal $f(t)$ can be written in terms of the two-time correlation function (Wiener-Khinchin theorem),

$$\mathrm{PSD}_f(\omega) = \int_{-\infty}^{\infty}\mathrm{d}\tau e^{i\omega\tau}R_f(\tau), \quad R_f(\tau) = \langle f(t)f(t-\tau)\rangle$$

For a discrete stepping motor, $v_{\rm m}(t) = \sum\limits_{n=-\infty}^{\infty} a\delta(t-t_n)$, in steady state we can thus write ($v = v_m$)

$$\begin{aligned}
\mathrm{PSD}_v(\omega) &= \int \mathrm{d}\tau e^{i\omega\tau} R_{v_{\rm m}}(\tau) = \int \mathrm{d}\tau\, e^{i\omega\tau}\langle v(t)v(t-\tau)\rangle \\
&= \lim_{T\to\infty}\frac{1}{T}\int_0^T \mathrm{d}t \int \mathrm{d}\tau\, e^{i\omega\tau}\langle v(t)v(t-\tau)\rangle \\
&= \lim_{T\to\infty}\frac{a^2}{T}\sum_{n,m=-\infty}^{\infty}\int_{-\infty}^{\infty} e^{i\omega\tau}\langle\delta(t_n-t_m-\tau)\rangle \mathrm{d}\tau \\
&= \lim_{T\to\infty}\frac{a^2}{T}\sum_{n,\Delta n=-\infty}^{\infty}\int_{-\infty}^{\infty} e^{i\omega\tau}\langle\delta(\Delta t_{\Delta n}-\tau)\rangle \mathrm{d}\tau \\
&= \lim_{T\to\infty}\frac{a^2}{T}\frac{T}{\langle\Delta t_1\rangle}\sum_{\Delta n=-\infty}^{\infty}\int_{-\infty}^{\infty} e^{i\omega\tau}\langle\delta(\Delta t_{\Delta n}-\tau)\rangle \mathrm{d}\tau \\
&= \frac{a^2}{\langle\Delta t_1\rangle}\sum_{\Delta n=-\infty}^{\infty}\int_{-\infty}^{\infty} e^{i\omega\tau}\langle\delta(\Delta t_{\Delta n}-\tau)\rangle \mathrm{d}\tau \\
&= \frac{a^2}{\langle\Delta t_1\rangle}\sum_{\Delta n=-\infty}^{\infty}\langle e^{i\omega\Delta t_{\Delta n}}\rangle \\
&= \frac{a^2}{\langle\Delta t_1\rangle}\left(1+\sum_{\Delta n=1}^{\infty}(\langle e^{i\omega\Delta t_{\Delta n}}\rangle + \langle e^{-i\omega\Delta t_{\Delta n}}\rangle)\right) \\
&= \frac{a^2}{\langle\Delta t_1\rangle}(1+Q(\omega)+Q(-\omega)),
\end{aligned}$$

where $\Delta n = m-n$, $\Delta t_{\Delta n} = t_n - t_m$, and $\frac{T}{\langle\Delta t_1\rangle}$ is the number of steps in time T, $Q(\omega) = \sum_{\Delta n=1}^{\infty} q_{\Delta n}(\omega)$, and $q_{\Delta n}(\omega) = \langle e^{i\omega\Delta t_{\Delta n}}\rangle$. This general formula applies to any stepping motor characterized by a $Q(\omega)$ according to,

$$\begin{aligned}
q_{\Delta n}(\omega) &= \langle e^{i\omega\Delta t_{\Delta n}}\rangle \\
&= \int_0^{\infty} P_{\Delta n}(\Delta t) e^{i\omega\Delta t}\mathrm{d}\Delta t.
\end{aligned}$$

For a Poisson process taking Δn identical sequential chemical steps, each taking an average duration of τ_0, the completion time Δt is described by the Gamma distribution [1],

$$P_{\Delta n}(\Delta t) = \frac{\Delta t^{\Delta n-1}}{(\Delta n-1)!\tau_0^{\Delta n}} e^{-\Delta t/\tau_0}, \tag{S15}$$

and

$$Q(\omega) + Q(-\omega) + 1 = 1.$$

If instead we have a stepping motor that takes m exponential sub-steps of average duration τ_0/m, the probability is now

$$P_{m\Delta n}(\Delta t) = \frac{\Delta t^{(m\Delta n-1)}}{(m\Delta n-1)!(\tau_0/m)^{m\Delta n}} e^{-(\Delta t/(\tau_0/m))}. \tag{S16}$$

With this we have

$$\begin{aligned} q_{\Delta n}(\omega) &= \langle e^{i\omega\Delta t_{\Delta n}}\rangle = \int_0^\infty P_{m\Delta n}(\Delta t) e^{i\omega\Delta t} \mathrm{d}\Delta t \\ &= \int_0^\infty \frac{\Delta t^{(m\Delta n-1)}}{(m\Delta n-1)!\,(\tau_0/m)^{m\Delta n}} e^{-\Delta t/(\tau_0/m)} e^{i\omega\Delta t} \mathrm{d}\Delta t \\ &= \frac{1}{(1-i\omega\tau_0/m)^{m\Delta n}} \end{aligned}$$

which can be summed to yield

$$Q(\omega) = \sum_{\Delta n=1}^{\infty} q_{\Delta n}(\omega) = \sum_{\Delta n=1}^{\infty} \frac{1}{(1-i\omega\tau_0/m)^{m\Delta n}} = \frac{1}{(1-i\omega\tau_0/m)^m - 1}$$

and

$$Q(\omega) + Q(-\omega) + 1 = \frac{1}{(1-i\omega\tau_0/m)^m - 1} + \frac{1}{(1+i\omega\tau_0/m)^m - 1} + 1$$

From this we have

$$\mathrm{PSD}_{v_\mathrm{m}} = \frac{a^2}{\tau_0}\alpha(\omega) = d\langle v_\mathrm{m}\rangle\alpha(\omega),$$

where

$$\alpha(\omega) = \left(1 + \frac{1}{(1-i\omega\tau_0/m)^m - 1} + \frac{1}{(1+i\omega\tau_0/m)^m - 1}\right). \tag{S17}$$

2 Comparison to time domain dwell time analysis

For the process where m "hidden" identical sequential chemical steps (rate k), the time τ to complete the m steps is distributed according the the Gamma distribution

$$p_m(\tau) = \frac{k^m \tau^{m-1}}{(m-1)!} e^{-k\tau}. \tag{S18}$$

Thus, fitting experimentally measured dwell time distributions to Equation (S18) allows the determination of the number of sequential steps, assuming the rate constants for each step are comparable [1–4]. In a scenario where the signal to noise allows resolution of individual mechanical steps, this analysis provides the number of rate-limiting biochemical processes per mechanical step. For experiments in which individual mechanical steps are not resolved, this analysis may be applied on an arbitrary distance Δx over which the dwell time can be reliably measured, thereby yielding the number of rate-limiting biochemical and mechanical steps over this distance [5, 6]. In case the experimental dwell time distribution differs from the one provided by Equation (S18), its shape can be used to suggest and build a more complete kinetic model for the motor [7, 8]. On the other hand, this treatment does not provide a way to measure or estimate the stiffness of the system.

This analysis method was applied to the simulations described in Materials and Methods. A dwell time window was defined as three times the estimated step size. For each trace, dwell times were calculated as the time to traverse the dwell time window. A normalized histogram of dwell times was fit with a gamma function, as per Equation (S18). The step size of the motor a was then calculated from the rate constant from the fit. Errors were calculated by performing 50 simulations with input parameters which matched the fit parameters of each of the experimental motors, then calculating the mean error of a over all the simulations. The results are shown in Fig S3.

We emphasize that the results shown in Fig 2 and Fig S3 are highly dependent upon the characteristics of both the motor and the experimental measurement. For example, the time domain dwell time analysis shows improved recovery of the step size for larger window sizes, given an infinitely long trace. For finite traces, a larger window size yields fewer points to fit Equation (S18), so there exists an optimal window size. For the parameters used in these simulations, both the time domain analysis of positions fluctuations and the frequency domain analysis of speed fluctuations show better accuracy and precision in the recovery of the motor step size.

3 Figures

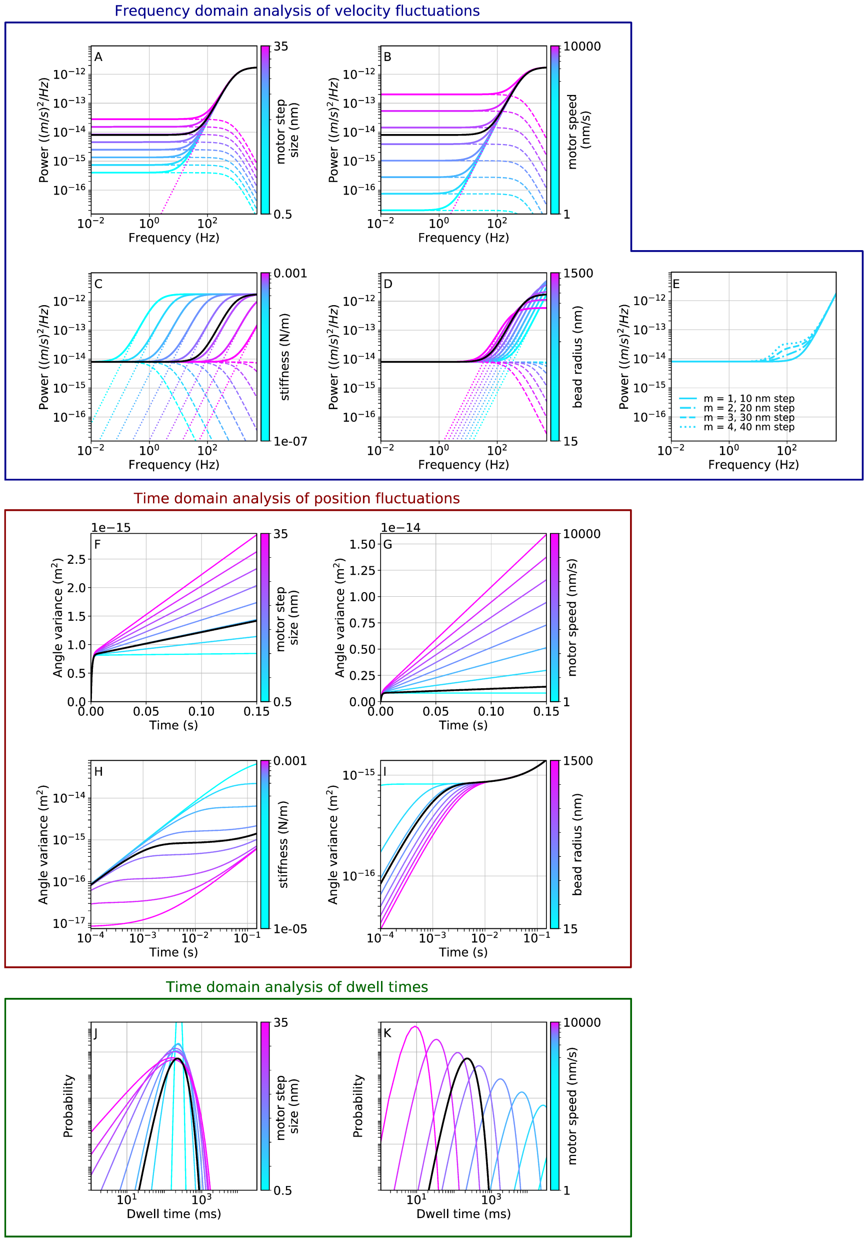

Figure S1: Demonstration of the theory for the two analysis methods presented in the manuscript as well as the time domain dwell time analysis discussed in SI. A-E) Frequency domain analysis of velocity fluctuations, F-I) Time domain analysis of position fluctuations, J-K) Time domain analysis of dwell times. For comparison, the black line in each subplot represents a 1 μm bead translocated at a speed of 400 nm/s by a motor which takes 10 nm steps, and a torsional hook stiffness of $1{\cdot}10^{-5}$ N/m. Each subplot varies a single variable from this standard, either linear or log-spaced, as shown and labeled by the colorbar on the right of the legend. Plot (E) shows the affects of the number of kinetic states for a 60 nm bead.

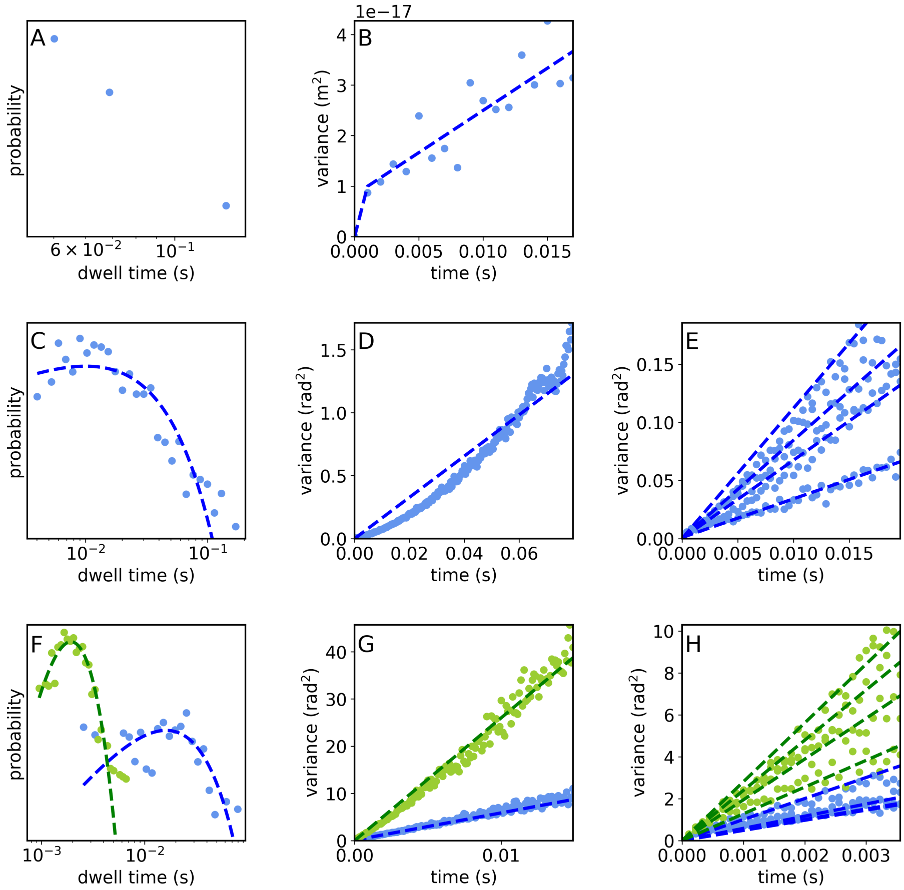

Figure S2: Experimental data (points) and fits (dashed lines) of the three molecular motors presented in Fig 3 of the manuscript, analyzed with the time domain analysis of dwell times and time domain analysis of position fluctuations. A) The length of the kinesin trace does not enable a fit to the dwell time data. B) time domain analysis of position fluctuations of kinesin yields a step size of 4.4 $\pm$ 0.9 nm and a stiffness of (1.0 $\pm$ 0.3)$\cdot 10^{-3}$ N/m. C) The dwell time analysis of the BFM yields a step size of 22 $\pm$ 3 degrees D) The time domain analysis of position fluctuations of the BFM yields a step size of 38 $\pm$ 2 degrees and a stiffness larger than can be reliably fit (greater than 300 pN nm/rad for the experimental conditions, as determined via simulation, not shown). E) The same analysis as in (D), but performed on four non-overlapping subsections of the trace, yielding step sizes of 15-18 degrees. The concavity in (D) arises from changes in motor speed. F) Dwell time analysis of F_1-ATPase yields a step size of 127 $\pm$ 11 degrees and 35 $\pm$ 8 degrees at low [ATP] (blue) and high [ATP] (green), respectively. G) Time domain analysis of position fluctuations of F_1 yields a step size of 132 $\pm$ 14 degrees and 50 $\pm$ 12 degrees at low [ATP] (blue) and high [ATP] (green), respectively. The stiffness is too high to be reliably fit (greater than 1 pN nm/rad for the experimental conditions, as determined via simulation, not shown). H) The same analysis as in (G), but performed on four non-overlapping subsections of the trace, yielding step sizes of 116-220 and 25-55 at low and high [ATP], respectively.

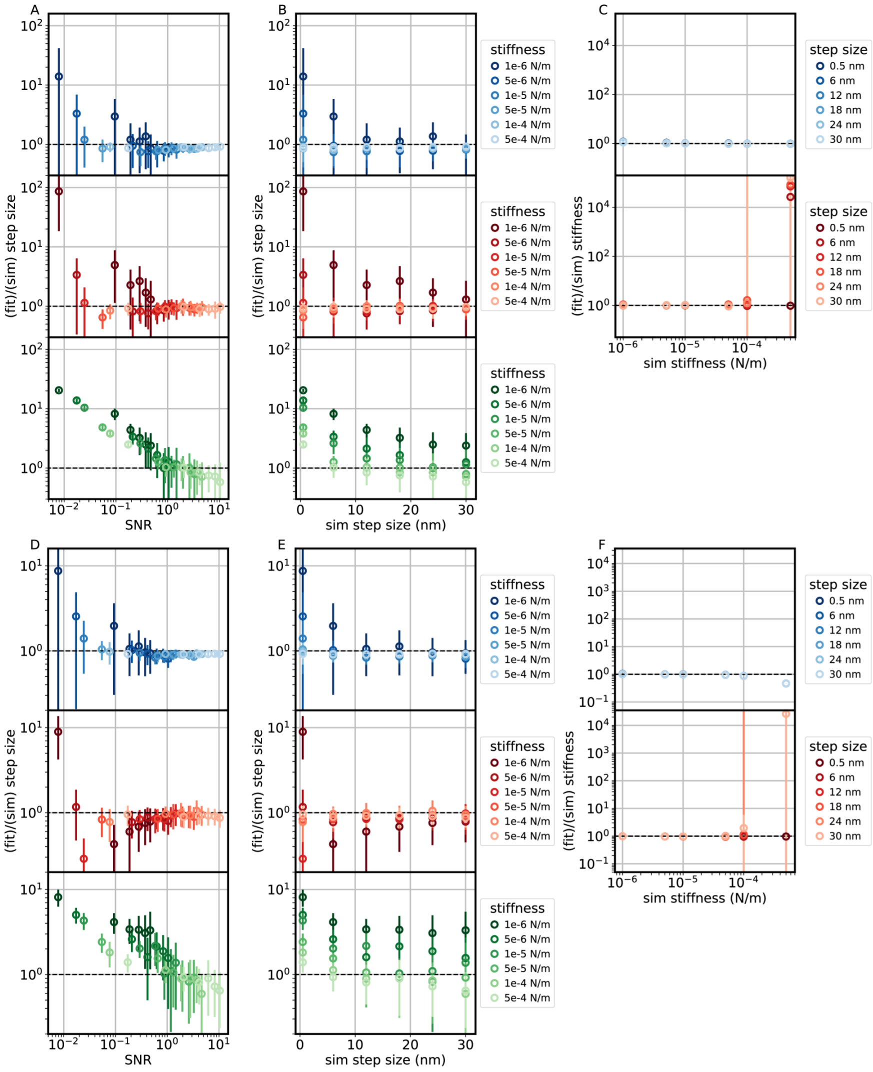

Figure S3: The performance of the frequency domain analysis of velocity fluctuations (blue) and time domain analysis of position fluctuations (red), compared to the time domain analysis of dwell times (green, Equation (S18)) on simulated traces of a linear motor driving A-C) a 1 μm bead at 400 nm/s or D-F) a 30 nm bead at 400 nm/s with step size and stiffness as labeled. A,D) Ratio of the extracted step size to the simulated step size as a function of the signal to noise ratio (SNR), defined as the simulated step size divided by the standard deviation of the difference between the bead position and motor position. B,E) Ratio of the extracted step size to the simulated step size as a function of the simulated step size. C,F) Ratio of the fit stiffness to the simulated stiffness as a function of the simulated stiffness. The dashed black lines represent perfect recovery of the input parameters. Points and error bars represent mean and standard deviation over 30 simulations (2 s each) per point.

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