Supporting Information: Quantifying the Heat Dissipation from a Molecular Motor's Transport Properties in Nonequilibrium Steady States

1. DERIVATION OF THE THIRD DEGREE POLYNOMIAL DEPENDENCE OF D ON V.

Here, we show a polynomial dependence of D on V using a few specific examples of the N-state periodic reaction model [1] whose reaction scheme is demonstrated in Fig. 1.

(N=1)-state kinetic model

When N=1, the master equation to solve is:

$$\dot{\pi}_\mu(t) = u_1\pi_{\mu-1}(t) + w_1\pi_{\mu+1}(t) - (u_1+w_1)\pi_\mu(t), \quad \text{(S1)}$$

where $\pi_\mu(t)$ is the probability of motor being in the μ-th reaction cycle at time t. Using generating function $F(z,t) = \sum_{\mu=-\infty}^{\infty} z^\mu \pi_\mu(t)$ with $\pi_\mu(0) = \delta_{\mu,0}$ [2], the master equation is written in terms of $F(z,t)$ as

$$\partial_t F(z,t) = \left(u_1 z + \frac{w_1}{z} - (u_1+w_1)\right) F(z,t)$$

$$F(z,t) = e^{\left(u_1 z + \frac{w_1}{z} - (u_1+w_1)\right)t}. \quad \text{(S2)}$$

Now, it is straightforward to obtain the mean velocity (V) and diffusion constant (D) using $\partial_z \log F(z,t)|_{z=1} = \langle\mu(t)\rangle$ and $\partial_z^2 \log F(z,t)|_{z=1} = \langle\mu^2(t)\rangle - \langle\mu(t)\rangle^2 - \langle\mu(t)\rangle$, where $\mu(t)$ is the number of steps taken by the molecular motor until time t.

$$V \equiv \lim_{t\to\infty} \frac{d_0\langle\mu(t)\rangle}{t} = d_0(u_1 - w_1) \quad \text{(S3)}$$

and

$$D \equiv \lim_{t\to\infty} \frac{d_0^2(\langle\mu^2(t)\rangle - \langle\mu(t)\rangle^2)}{2t} = \frac{d_0^2(u_1 + w_1)}{2}. \quad \text{(S4)}$$

Provided that only u_1 changes (for example by increasing ATP concentration) while w_1 remains constant. elimination of u_1 from $V(u_1)$ and $D(u_1)$ relates D to V as

$$D(V) = D_0 + \frac{d_0}{2}V \quad \text{(S5)}$$

where

$$D_0 \equiv d_0^2 w_1, \quad \text{(S6)}$$

showing that for (N=1)-state kinetic model, D is linear in V.

(N=2)-state kinetic model

For the (N=2)-kinetic model [3],

$$V = d_0 \frac{u_1 u_2 - w_1 w_2}{u_1 + u_2 + w_1 + w_2} \quad \text{(S7)}$$

and

$$D = \frac{d_0^2}{2}\left[\frac{u_1 u_2}{w_1 w_2} + 1 - 2\left(\frac{u_1 u_2}{w_1 w_2} - 1\right)^2 \frac{w_1 w_2}{\sigma^2}\right]\frac{w_1 w_2}{\sigma} \quad \text{(S8)}$$

where $\sigma = u_1 + u_2 + w_1 + w_2$.

Then, $D = D(V)$ is obtained by eliminating u_1 between Eq.S7 and Eq.S8:

$$D(v)/d_0^2 = \left(\frac{u_2}{\kappa+1}\right) + \frac{1}{2}\left(\frac{\kappa-1}{\kappa+1}\right)u_2 v - \left(\frac{u_2}{\kappa+1}\right)\frac{u_2^2}{w_1 w_2}v^2 + \left(\frac{u_2}{\kappa+1}\right)\frac{u_2^2}{w_1 w_2}v^3 \quad \text{(S9)}$$

where $\kappa \equiv \frac{u_2(u_2+w_1+w_2)}{w_1 w_2}$, and $v \equiv V/V_{max}$ ($0 \le v \le 1$) with $V_{max} = d_0 u_2$. Eq.S9 confirms that D is a third order polynomial in V.

Incidentally, the (N=2)-kinetic model is reduced to the Michaelis-Menten equation by setting $u_1 = u_1^o[S]$ and $w_2 = 0$.

$$V = d_0 \frac{u_1 u_2}{u_1 + u_2 + w_1} = \frac{V_{max}[S]}{K_M + [S]} \quad \text{(S10)}$$

where $K_M = (u_2 + w_1)/u_1^o$, and

$$D(v) = D_{max}v\left[1 - 2\phi v + 2\phi v^2\right] \quad \text{(S11)}$$

with $\phi \equiv \frac{k_{cat}}{u_1^o K_M}$. Note that $D \leq D_{max} = d_0 V_{max}/2$ and that for $D(v)$ to be positive for all the range of v, the parameter ϕ should be in a rather narrow range of $0 \leq \phi \leq 2$.

General case: N-state kinetic model

The above two examples of one-dimensional hopping model was extended to the N-state kinetic model by Derrida [1]. He obtained exact expressions for the mean ve-

locity ($V^{(D)}$) and diffusion constant ($D^{(D)}$), where the superscript (D) refers to Derrida's, in terms of the rate constants $\{u_n\}$ and $\{w_n\}$. Derrida's expression for $V^{(D)}$ and $D^{(D)}$ are related to V and D as $V \equiv (d_0/N)V^{(D)}$ and $D \equiv (d_0^2/N^2)D^{(D)}$.

$$V^{(D)} = \frac{N}{\sum_{n=1}^N r_n} \left(1 - \frac{\prod_{n=1}^N w_n}{\prod_{n=1}^N u_n} \right) \qquad (S12)$$

and

$$D^{(D)} = \frac{1}{\left(\sum_{n=1}^N r_n\right)^2} \left[V^{(D)} \sum_{n=1}^N q_n \sum_{i=1}^N i r_{n+i} + N \sum_{n=1}^N u_n q_n r_n \right] - V^{(D)} \frac{N+2}{2} \qquad (S13)$$

where $r_n = \frac{1}{u_n}\left[1 + \sum_{i=1}^{N-1} \prod_{j=1}^i \frac{w_{n+j-1}}{u_{n+j}}\right]$, and $q_n = \frac{1}{u_n}\left[1 + \sum_{i=1}^{N-1} \prod_{j=1}^i \frac{w_{n-j}}{u_{n-j}}\right]$ with periodic boundary conditions $u_{n+N} = u_n$, and $w_{n+N} = w_n$. $D^{(D)} = D^{(D)}(V^{(D)})$ is obtained by eliminating u_1 between Eq.S12 and Eq.S13. For that, we first express various terms in Eq.S13 in terms of u_1:

$$\sum_{n=1}^N r_n = \frac{A}{u_1} + B,$$

$$\sum_{n=1}^N q_n \sum_{i=1}^N i r_{n+i} = \frac{\alpha}{u_1^2} + \frac{\beta}{u_1} + \gamma,$$

$$\sum_{n=1}^N u_n q_n r_n = \frac{\xi}{u_1^2} + \frac{\eta}{u_1} + \zeta. \qquad (S14)$$

where A, $B \left(= \sum_{n=2}^N \frac{1}{u_n}\left[1 + \sum_{i=1}^{N-n} \prod_{j=1}^i \frac{w_{n+j-1}}{u_{n+j}}\right]\right)$, α, β, γ, ξ, η, and ζ are all positive constants independent of u_1. Next, $\sum_{n=1}^N r_n$ in Eq.S14 substituted to Eq.S12 gives

$$V^{(D)} = N\frac{1 - C/u_1}{A/u_1 + B}, \qquad (S15)$$

where $C \left(= \prod_{n=1}^N w_n / \prod_{n=2}^N u_n\right)$, and u_1 is expressed in terms of $V^{(D)}$

$$\frac{1}{u_1} = \frac{1 - B\frac{V^{(D)}}{N}}{C + A\frac{V^{(D)}}{N}}. \qquad (S16)$$

Finally, with Eqs.S14 and S16, we show that $D^{(D)}$ (Eq.S13) can be expressed as a third degree polynomial in $V^{(D)}$

$$D^{(D)} = V^{(D)}\frac{\left[\alpha(1 - B\frac{V^{(D)}}{N})^2 + \beta(1 - B\frac{V^{(D)}}{N})(A\frac{V^{(D)}}{N} + C) + \gamma(A\frac{V^{(D)}}{N} + C)^2\right]}{(A + BC)^2}$$

$$+ N\frac{\xi(1 - B\frac{V^{(D)}}{N})^2 + \eta(1 - B\frac{V^{(D)}}{N})(A\frac{V^{(D)}}{N} + C) + \zeta(A\frac{V^{(D)}}{N} + C)^2}{(A + BC)^2} - V^{(D)}\frac{N+2}{2}$$

$$= z_0 + z_1 V^{(D)} + z_2 (V^{(D)})^2 + z_3 (V^{(D)})^3$$

$$D = \alpha_0 + \alpha_1 V + \alpha_2 V^2 + \alpha_3 V^3. \qquad (S17)$$

with $\alpha_i = (d_0/N)^{2-i} z_i$, and

$$z_0 = \frac{(\xi + \eta C + \zeta C^2)}{(A + BC)^2}$$

$$z_1 = \left[\frac{(\alpha + \beta C + \gamma C^2) + (-2\xi B + \eta(A - BC) + 2\zeta AC)}{(A + BC)^2} - \frac{N + 2}{2}\right]$$

$$z_2 = \frac{(-2\alpha B + \beta(A - BC) + 2\gamma AC) + (\xi B^2 - \eta AB + \zeta A^2)}{(A + BC)^2}$$

$$z_3 = \frac{(\alpha B^2 - \beta AB + \gamma A^2)}{(A + BC)^2}.$$

Alternative derivation of $D(V)$

In addition to Derrida's result [1], the sign of α_i can be determined by deriving the relation between $V^{(D)}$ and $D^{(D)}$ using the result in ref. [4]. From Eq.(23) in ref. [4],

$$V^{(D)} = -i\frac{c_0'}{c_1} \tag{S18}$$

where $c_0' = iN(\prod_{n=1}^{N} u_n - \prod_{n=1}^{N} w_n)$ and $c_1 = c_1(\{u_n\}, \{w_n\})$. By combining two expressions of $V^{(D)}$, Eq.S12 and Eq.S18, we get

$$\begin{aligned} c_1 &= \prod_{n=1}^{N} u_n \times \sum_{m=1}^{N-1}\left[\frac{1}{u_m}\left(1 + \sum_{i=1}^{N-1}\prod_{j=1}^{i}\frac{w_{m+j}}{u_{m+j}}\right)\right] \\ &= u_1 \prod_{n=2}^{N} u_n \left[\frac{1}{u_1}\left(1 + \sum_{i=1}^{N-1}\prod_{j=1}^{i}\frac{w_{1+j}}{u_{1+j}}\right) + \sum_{m=2}^{N-1}\frac{1}{u_m}\left(1 + \sum_{i=1}^{N-1}\prod_{j=1}^{i}\frac{w_{m+j}}{u_{m+j}}\right)\right] \\ &= \mathcal{A}u_1 + \mathcal{B} \end{aligned} \tag{S19}$$

where $\mathcal{A}$ and $\mathcal{B}$ are constants depending on $(u_2, \ldots, u_N)$ and $(w_1, w_2, \ldots w_N)$. Eq.(S19) substituted to Eq.(S18) gives

$$V^{(D)} = N\frac{u_1 \prod_{n=2}^{N} u_n - \prod_{n=1}^{N} w_n}{\mathcal{A}u_1 + \mathcal{B}} \tag{S20}$$

and hence u_1 can be written as

$$u_1 = \frac{\mathcal{B}V^{(D)} + N\prod_{n=1}^{N} w_n}{N\prod_{n=2}^{N} u_n - \mathcal{A}V^{(D)}}. \tag{S21}$$

From Eq.S16 and Eq.S21, $\mathcal{A} = B\prod_{n=2}^{N} u_n$ and $\mathcal{B} = A\prod_{n=2}^{N} u_n$ where A and B are the same constants used in Eq.S15. Now using Eq.(S19), Eq.(S16), and general

expression of $D^{(D)}$ from Eq.(24) of ref. [4], we have

$$\begin{aligned} D^{(D)} &= \frac{c_0'' - 2c_2(V^{(D)})^2}{2c_1} \\ &= \frac{c_0'' - 2c_2(V^{(D)})^2}{2(\frac{B}{\prod_{n=2}^{N} u_n}u_1 + \frac{A}{\prod_{n=2}^{N} u_n})} \\ &= \frac{(c_0'' - 2c_2(V^{(D)})^2)(\prod_{n=2}^{N} u_n(N - BV^{(D)}))}{2N(A + CB)} \end{aligned} \tag{S22}$$

where $c_0'' = N^2(u_1\prod_{n=2}^{N} u_n + \prod_{n=1}^{N} w_n) = N^2(\prod_{n=2}^{N} u_n)(u_1 + C)$, and $c_2 = \beta_1 u_1 + \beta_2$ where β_1 and β_2 are positive constants depending on $(u_2, u_3, \cdots, u_N)$ and $(w_1, w_2, \cdots, w_N)$ (see Eq.(53, 54) of ref. [4]). Since $u_1(N - BV^{(D)}) = NC + AV^{(D)}$ (Eq.S15), we get

$$D^{(D)} = \frac{1}{2} \frac{(c_0'' - 2c_2(V^{(D)})^2)((N - BV^{(D)})) \prod_{n=2}^{N} u_n}{NA + NCB}$$

$$= \frac{1}{2} \frac{(N^2(\prod_{n=2}^{N} u_2)(u_1 + C) - 2(\beta_1 u_1 + \beta_2)(V^{(D)})^2)((N - BV^{(D)})) \prod_{n=2}^{N} u_n}{NA + NCB}$$

$$= \frac{\prod_{n=2}^{N} u_n}{2} \frac{\left(N^2(\prod_{n=2}^{N} u_2)(NC + AV^{(D)} + C(N - BV^{(D)})) - 2(\beta_1(NC + AV^{(D)}) + \beta_2(N - BV^{(D)}))(V^{(D)})^2 \right)}{NA + NCB}$$

$$= z_0 + z_1 V^{(D)} + z_2 (V^{(D)})^2 + z_3 (V^{(D)})^3 \tag{S23}$$

where

$$z_0 = \frac{N^2(\prod_{n=2}^{N} u_n)^2 C}{A + BC} > 0$$

$$z_1 = \frac{N(\prod_{n=2}^{N} u_n)^2 (A - BC)}{2(A + BC)}$$

$$z_2 = -\frac{(\prod_{n=2}^{N} u_n)(\beta_1 C + \beta_2)}{A + BC} < 0$$

$$z_3 = \frac{(\prod_{n=2}^{N} u_n)(-\beta_1 A + \beta_2 B)}{N(A + BC)}.$$

It is obvious that $z_0 > 0$ and $z_2 < 0$ since A, B, C, β_1, and β_2 are all positive constants.

2. THE 1D HOPPING MODEL WITH A FINITE PROCESSIVITY

Because of a probability of being dissociated from microtubules, kinesin motors display a finite processivity. However, since the mean velocity and diffusion constant are calculated from the trajectories that remain on the track, the expressions of V and D in terms of the rate constants are unchanged. To make this point mathematically more explicit, we consider the master equation assuming a constant dissociation rate k_d from each chemical state.

$$\frac{dP_{\mu,n}(t)}{dt} = u_{n-1}P_{\mu,n-1}(t) + w_n P_{\mu,n+1}(t)$$
$$- (u_n + w_{n-1} + k_d)P_{\mu,n}(t) \tag{S24}$$

where $P_{\mu,n}(t)$ is the probability of being in the n-th chemical state at the μ-th reaction cycle. The probability of the motor remaining on the track (survival probability of motor) is

$$S(t) \equiv \sum_{\mu=\infty}^{\infty} \sum_{n=1}^{N} P_{\mu,n}(t) = e^{-k_d t}. \tag{S25}$$

The expectation value of an observable, which can be used to calculate $\langle x(t) \rangle$ or $\langle x^2(t) \rangle$, is expressed as

$$\langle A(t) \rangle = \sum_{\mu=-\infty}^{\infty} \sum_{n=1}^{N} \Phi_{\mu,n}(t) A(\mu(t)) \tag{S26}$$

with a probability density function renormalized with respect to the survival probability

$$\Phi_{\mu,n}(t) \equiv \frac{P_{\mu,n}(t)}{S(t)} = P_{\mu,n}(t)e^{k_d t}. \tag{S27}$$

Incidentally, $\Phi_{\mu,n}(t)$ satisfies the following master equation.

$$\frac{d\Phi_{\mu,n}(t)}{dt} = u_{n-1}\Phi_{\mu,n-1}(t) + w_n \Phi_{\mu,n+1}(t) - (u_n + w_{n-1})\Phi_{\mu,n}(t), \tag{S28}$$

which is identical to Eq.1, but now the probability of interest is explicitly confined to the ensemble of trajectories remaining on the track. For an arbitrary value of k_d and for any N, the expressions of V, D, and Eq.S17 remain unchanged except that the range of ensemble is specific to the motor trajectories remaining on the track. Furthermore, the expression of $\dot{Q}$, which depends only on V and rate constants, remains identical in the presence of detachment (finite $k_d > 0$). Therefore, our formalisms remain valid for motors with a finite processivity.

3. MAPPING THE MASTER EQUATION FOR N-STATE KINETIC MODEL ONTO LANGEVIN AND FOKKER-PLANCK EQUATIONS

The master equation (Eq.S1) can be mapped onto a Langevin equation for position $x(t)$ as

$$\dot{x}(t) = V + \sqrt{2D}\eta(t) \tag{S29}$$

where for (N=1)-state model $V = d_0(u_1 - w_1)$ and $D = d_0^2/2 \times (u_1 + w_1)$ as in Eqs.S3 and S4, and $P[\eta(t)] \propto$

$\exp\left(-\frac{1}{2}\int_0^t d\tau\eta^2(\tau)\right)$. Then, with the transition probability (propagator),

$$P(x_{t+\epsilon}|x_t) = \left(\frac{1}{4\pi D\epsilon}\right)^{1/2} e^{-\frac{\{x_{t+\epsilon}-x_t-\epsilon V\}^2}{4D\epsilon}}, \tag{S30}$$

where $x_t \equiv x(t)$, and starting from an initial condition, $P[x(0)] = \delta[x(0) - x_0]$, it is straightforward to obtain the position of motor at time t:

$$P[x(t)] = \int dx_0 \int dx_\epsilon \cdots \int dx_{t-\epsilon} P(x_t|x_{t-\epsilon}) \cdots P(x_{2\epsilon}|x_\epsilon)P(x_\epsilon|x_0)P(x_0)$$
$$= \left(\frac{1}{4\pi Dt}\right)^{1/2} \exp\left(-\frac{(x(t) - x_0 - Vt)^2}{4Dt}\right)$$
$$= \left(\frac{1}{2\pi d_0^2(u_1 + w_1)t}\right)^{1/2} \exp\left(-\frac{[x(t) - x_0 - d_0(u_1 - w_1)t]^2}{2d_0^2(u_1 + w_1)t}\right), \tag{S31}$$

where we plugged V and D from Eqs.S3, S4 for (N=1)-state kinetic model in the last line. Unlike the normal Langevin equation, where the noise strength determined by FDT is associated with an ambient temperature ($\sim \sqrt{T}$), the noise strength in Eq.S29 is solely determined by the forward and backward rate constants, which fundamentally differs from the Brownian motion of a thermally equilibrated colloidal particle in a heat bath.

Next, Fokker-Planck equation follows from Eq.S29,

$$\partial_t P(x, t) = D\partial_x^2 P(x, t) - V\partial_x P(x, t)$$
$$= -\partial_x j(x, t) \tag{S32}$$

with the probability current being defined as

$$j(x, t) = -D\partial_x P(x, t) + VP(x, t). \tag{S33}$$

Then, *mean local velocity* $v(x, t) \equiv j(x, t)/P(x, t)$ is defined

$$v(x, t) = V - D\partial_x \log P(x, t). \tag{S34}$$

In order to relate this definition of the mean local velocity to heat dissipated from the molecular motor moving along microtubules in a NESS, we consider γ_{eff}, *an effective friction coefficient*, and introduce a nonequilibrium potential $\phi(x) \equiv -\log P^{ss}(x)$ [5, 6]. By integrating the both side of Eq.S34 in a NESS with respect to the displacement corresponding to a single step, we obtain

$$\int_x^{x+d_0} \gamma_{\text{eff}} v^{ss}(x)dx = \gamma_{\text{eff}} Vd_0 + \gamma_{\text{eff}} D(\phi(x + d_0) - \phi(x)). \tag{S35}$$

Following the literature on NESS thermodynamics [5–7], we endowed each term of Eq.S35 with its physical meaning. (i) housekeeping heat:

$$Q_{hk} = \int_x^{x+d_0} \gamma_{\text{eff}} v(x)dx, \tag{S36}$$

(ii) total heat:

$$Q = \gamma_{\text{eff}} Vd_0 \tag{S37}$$

and (iii) excess heat:

$$Q_{ex} = -\gamma_{\text{eff}} D(\phi(x + d_0) - \phi(x)). \tag{S38}$$

Eqs.S36, S37, and S38 satisfy

$$Q_{hk} = Q - Q_{ex}. \tag{S39}$$

and in fact $Q_{ex} = 0$ because of the periodic boundary condition implicit to our problem of molecular motor, which leads to $\phi(x + d_0) = \phi(x)$. Hence,

$$Q_{hk} = Q = \gamma_{\text{eff}} Vd_0. \tag{S40}$$

Although we introduced the effective friction coefficient γ_{eff} in Eq.S35 to define the heats produced at nonequilibrium, Eq.S40 finally allows us to associate γ_{eff} with other physically well-defined quantities.

$$\gamma_{\text{eff}} = \frac{Q_{hk}}{Vd_0} = \frac{k_B T}{d_0^2(j_+ - j_-)} \log\left(\frac{j_+}{j_-}\right). \tag{S41}$$

Here, note that we for the first time introduced the temperature T, which was discussed neither in the master equation (Eq.S1) nor in the Langevin equation (Eq.S29). Of special note is that γ_{eff} does not remain constant, but depends on the steady state flux $j^{ss} = j = j_+ - j_-$. Similar to the effective diffusion constant of bacterium, D_{eff}, discussed in the main text, γ_{eff} is defined operationally. At equilibrium, when the detailed balance (DB) is established ($j_+ = j_- = j_0$), $\gamma_{\text{eff}}^{\text{DB}}$ approaches to:

$$\gamma_{\text{eff}}^{\text{DB}} = \lim_{j_+ \to j_-} \frac{k_B T}{d_0^2 (j_+ - j_-)} \log\left(\frac{j_+}{j_-}\right) \to \frac{k_B T}{d_0^2 j_0}. \quad (S42)$$

In fact, $D_0 = k_B T / \gamma_{\text{eff}}^{\text{DB}} = d_0^2 j_0$ satisfies the FDT for passive particle at thermal equilibrium, i.e., $k_B T = D_0 \gamma_{\text{eff}}^{\text{DB}}$. For ($N=1$)-state model, $D_0 = d_0^2 w_1$, which is identical to Eq.S6.

4. NONEQUILIBRIUM STEADY STATE THERMODYNAMICS.

To drive a system out of equilibrium, one has to supply a proper form of energy into the system. Molecular motors move in one direction because transduction of chemical free energy into conformational change is processed. Relaxation from a nonequilibrium state is accompanied with heat and entropy production. In the presence of external nonconservative force (chemical or mechanical force), the system reaches the nonequilibrium steady state. If one considers a Markov dynamics for microscopic state i, described by the master equation $\partial_t p_i(t) = -\sum_j (W_{ij} p_i(t) - W_{ji} p_j(t))$, the system relaxes to nonequilibrium steady state at long time, establishing time-independent steady state probability $\{p_i^{ss}\}$ for each state satisfying the zero flux condition $\sum_j (W_{ij} p_i^{ss} - W_{ji} p_j^{ss}) = 0$. A removal of the nonconservative force is led to further relaxation to the equilibrium ensemble, in which the detailed balance (DB) is (locally) established in every pair of the states such that $p_i^{eq} W_{ij} = p_j^{eq} W_{ji}$ for all i and j. An important feature of the equilibrium, which differentiates itself from NESS, is the condition of DB.

Over the decade, there have been a number of endeavors to better characterize the system out-of-equilibrium [5]. One of them is to define the heat and entropy production in the context of Master equation. The heat and entropy productions in reference to either steady state or equilibrium are defined to better characterize the process of interest. The aim is to associate the time dependent probability for state ($\{p_i(t)\}$) and transition rates between the states $\{W_{ij}\}$ with newly defined macroscopic thermodynamic quantities at nonequilibrium [8]. Here, we review NESS thermodynamics formalism developed by Ge and Qian [8].

For nonequilibrium relaxation processes one can consider three relaxation processes: (i) relaxation process of a system far-from-equilibrium (FFE) to a nonequilibrium steady state (NESS); (ii) relaxation process of a system far-from-equilibrium (FFE) to an equilibrium (EQ). (iii) relaxation process of a system in NESS to an equilibrium (EQ). To describe these relaxation processes using the probabilities for state, we introduce a phenomenological definition of an internal energy of state i at a steady state by $u_i^{ss} = -k_B T \log p_i^{ss}$, and at equilibrium by $u_i^{eq} = -k_B T \log p_i^{eq}$. Then the following thermodynamic quantities are defined either in reference to NESS or equilibrium.

First, the thermodynamic potentials are defined in reference to the NESS: the total energy $U(t) = \sum_i^N p_i(t) u_i^{ss}$; the total free energy $F(t) = U(t) - TS(t) = k_B T \sum_i^N p_i(t) \log (p_i(t)/p_i^{ss})$. Second, the thermodynamic potentials are defined in reference to the equilibrium: the total energy $U^{eq}(t) = \sum_i^N p_i(t) u_i^{eq}$; the total free energy $F^{eq}(t) = U^{eq}(t) - TS(t) = k_B T \sum_i^N p_i(t) \log (p_i(t)/p_i^{eq})$. In both cases, Gibbs entropy, $S(t) = -k_B \sum_i^N p_i(t) \log p_i(t)$, is defined as usual.

Next, the above definitions of generalized thermodynamic potentials, one can define the heat and entropy productions associated with the relaxation processes (i), (ii), (iii). The diagram in Fig.S1 depicts the relaxation processes mentioned here.
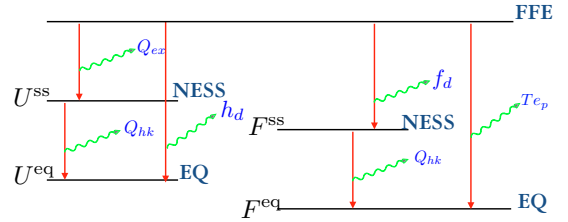

FIG. S1. A diagram illustrating the balances between various thermodynamic quantities discussed in the text. The curvy arrows denote the heat and entropy production from relaxation processes.

$-dF(t)/dt$ is the rate of entropy production in the relaxation from FFE to NESS,

$$\frac{dF(t)}{dt} \equiv -\dot{f}_d = -T\sum_{i>j}[W_{ij}p_i(t) - W_{ji}p_j(t)]\log\left[\frac{p_i(t)p_j^{ss}}{p_j(t)p_i^{ss}}\right]. \tag{S43}$$

and $-dU(t)/dt$ is the rate of heat production in the relaxation from FFE to NESS.

$$\frac{dU(t)}{dt} \equiv -\dot{Q}_{ex} = -\sum_{i>j}(W_{ij}p_i(t) - W_{ji}p_j(t))(u_i^{ss} - u_j^{ss})$$

$$= T\sum_{i>j}(W_{ij}p_i - W_{ji}p_j)\log\left(\frac{p_i^{ss}}{p_j^{ss}}\right). \tag{S44}$$

Similarly, $-dF^{eq}(t)/dt$ is the rate of entropy production during the relaxation to equilibrium,

$$\frac{dF^{eq}(t)}{dt} \equiv -T\dot{e}_p = -T\sum_{i>j}[W_{ij}p_i(t) - W_{ji}p_j(t)]\log\left[\frac{p_i(t)W_{ij}}{p_j(t)W_{ji}}\right] \tag{S45}$$

and $-dU^{eq}(t)/dt$ is the rate of heat production.

$$\frac{dU^{eq}(t)}{dt} \equiv -\dot{h}_d = -\sum_{i>j}(W_{ij}p_i(t) - W_{ji}p_j(t))(u_i^{eq} - u_j^{eq})$$

$$= T\sum_{i>j}(W_{ij}p_i - W_{ji}p_j)\log\left(\frac{W_{ij}}{W_{ji}}\right) \tag{S46}$$

where the condition of DB ($p_i^{eq}/p_j^{eq} = W_{ji}/W_{ij}$) was used to derive the last line. Furthermore, the heat production involved with the relaxation from NESS to equilibrium ($\dot{Q}_{hk}$), namely housekeeping heat which is introduced in NESS stochastic thermodynamics from the realization that maintaining NESS requires some energy, is defined by either using $\dot{Q}_{hk} = (-dF^{eq}(t)/dt) - (-dF(t)/dt) = T\dot{e}_p - \dot{f}_d$ or $\dot{Q}_{hk} = (-dU^{eq}(t)/dt) - (-dU(t)/dt) = \dot{h}_d - \dot{Q}_{ex}$. Explicit calculations using the representation of thermodynamic potential in terms of master equation lead to

$$\dot{Q}_{hk} = T\sum_{i>j}[W_{ij}p_i(t) - W_{ji}p_j(t)]\log\left[\frac{p_i^{ss}W_{ij}}{p_j^{ss}W_{ji}}\right]. \tag{S47}$$

Lastly, from the definition of Gibbs entropy ($S(t) = -k_B\sum_i p_i(t)\log p_i(t)$), or from the thermodynamic relationships $TdS/dt = dF/dt - dU/dt = dF^{eq}/dt - dU^{eq}/dt$, it is straightforward to show that

$$T\frac{dS}{dt} = \frac{dF}{dt} - \frac{dU}{dt} = \frac{dF^{eq}}{dt} - \frac{dU^{eq}}{dt}$$

$$= -T\sum_{i>j}[W_{ij}p_i(t) - W_{ji}p_j(t)]\log\left(\frac{p_i(t)}{p_j(t)}\right)$$

$$= \dot{h}_d - T\dot{e}_p. \tag{S48}$$

Now, with the various heat and entropy production defined from generalized potentials $F(t)$, $U(t)$, and $F^{eq}(t)$, $U^{eq}(t)$ ($\dot{f}_d$, $\dot{Q}_{ex}$, $T\dot{e}_p$, $\dot{h}_d$, and $\dot{Q}_{hk}$) we acquire two important balance laws in nonequilibrium thermodynamics:

$$T\dot{e}_p = \dot{f}_d + \dot{Q}_{hk}$$

$$\dot{h}_d = \dot{Q}_{hk} + \dot{Q}_{ex} \tag{S49}$$

Thus, (i) the total entropy production of a system, $T\dot{e}_p(= -dF^{eq}/dt)$, is contributed by the free energy dissipation due to the relaxation to NESS, $\dot{f}_d(= -dF/dt)$, and the housekeeping heat, $\dot{Q}_{hk}(= dF/dt - dF^{eq}/dt)$, that is required to maintain the NESS. (ii) The total heat production $\dot{h}_d(= -dU^{eq}/dt)$ of a system is decomposed into $\dot{Q}_{hk}(= dU/dt - dU^{eq}/dt)$ and the excess heat $\dot{Q}_{ex}(= -dU/dt)$. The diagram in Fig.S1 recapitulates the various heat and entropy production terms and their balance. When the system is already in NESS, then neither the production of entropy nor excess heat is anticipated ($\dot{f}_d = 0$, $\dot{Q}_{ex} = 0$), and hence it follows that the amount of heat, entropy, and housekeeping heat required to sustain NESS are identical ($T\dot{e}_p = \dot{Q}_{hk} = \dot{h}_d$).

In order to gain a better insight into the energy balance of molecular motor that operates in nonequilibrium steady state, we consider the dynamics of molecular motor systems by means of a cyclic Markov model and relate the essential parameters of the model with NESS
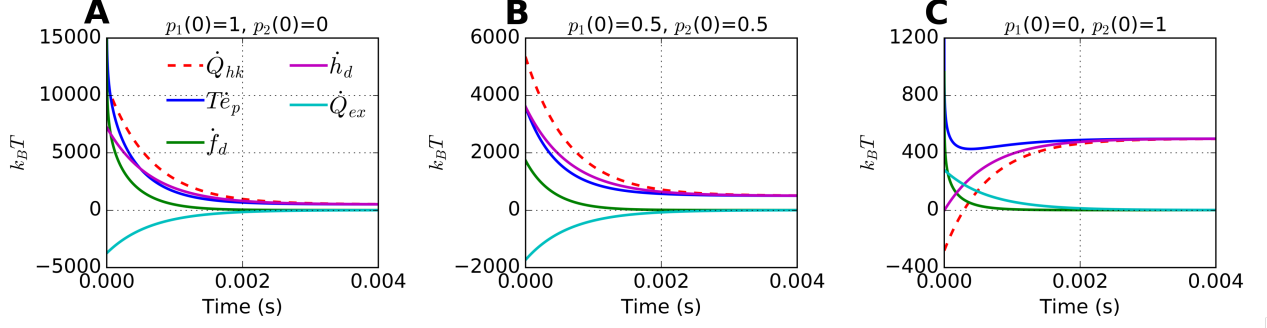
FIG. S2. Relaxation dynamics of various nonequilibrium thermodynamic quantities from far-from-equilibrium states calculated using (N=2)-state system. The parameters used for the plots are: $[\text{ATP}] = 1$ mM, $f = 1$ pN; $u_1^0 = 1.8\ s^{-1}\mu M^{-1}$, $u_2 = 108\ s^{-1}$, $w_1 = 6.0\ s^{-1}$, and $w_2 = 16\ s^{-1}$ at zero load; $\theta_1^+ = 0.135, \theta_2^+ = 0.035, \theta_1^- = 0.080$, and $\theta_2^- = 0.75$. Plots were made using three different initial conditions: **A.** $p_1(0) = 1, p_2(0) = 0$; **B.** $p_1(0) = 0.5, p_2(0) = 0.5$; and **C.** $p_1(0) = 0, p_2(0) = 1$.

thermodynamics. The thermodynamic quantities associated with nonequilibrium process $(\dot{f}_d, \dot{Q}_{hk}, T\dot{e}_p, \dot{h}_d, \dot{Q}_{ex})$ can be evaluated explicitly using $(N = 2)$-state Markov model; the time evolution of each state is given by $p_1(t) = p_1^{ss} + (p_1(0) - p_1^{ss})e^{-\sigma t}$ and $p_2(t) = p_2^{ss} - (p_1(0) - p_1^{ss})e^{-\sigma t}$

with $p_1^{ss} = (u_2 + w_1)/\sigma$, $p_2^{ss} = (u_1 + w_2)/\sigma$, and $\sigma = u_1 + u_2 + w_1 + w_2$. Using the conditions satisfied in 2-state model $(p_{1+2}(t) = p_1(t))$ $W_{12} = u_1$, $W_{21} = w_1$, $W_{23} = u_2$, $W_{32} = w_2$; otherwise $W_{ij} = 0$, we obtain

$$
\begin{aligned}
\frac{\dot{Q}_{hk}(t)}{T} &= [w_1 p_2(t) - u_1 p_1(t)] \log\left[\frac{(u_2 + w_1)w_1}{(u_1 + w_2)u_1}\right] + [w_2 p_1(t) - u_2 p_2(t)] \log\left[\frac{(u_1 + w_2)w_2}{(u_2 + w_1)u_2}\right] \\
&= \frac{u_1 u_2 - w_1 w_2}{u_1 + u_2 + w_1 + w_2} \log\left[\frac{u_1 u_2}{w_1 w_2}\right] - \lambda(p_1(0) - p_1^{ss})e^{-\sigma t} \\
&\xrightarrow{\sigma t \gg 1} (j_+ - j_-) \log\left(\frac{j_+}{j_-}\right) \geq 0
\end{aligned}
\tag{S50}
$$

where $\lambda = \left\{(u_1 + w_1) \log\left[\frac{(u_2+w_1)w_1}{(u_1+w_2)u_1}\right] - (u_2 + w_2) \log\left[\frac{(u_1+w_2)w_2}{(u_2+w_1)u_2}\right]\right\}$.

$$
\begin{aligned}
\dot{e}_p(t) &= [w_1 p_2(t) - u_1 p_1(t)] \log\left[\frac{p_2(t)w_1}{p_1(t)u_1}\right] + [w_2 p_1(t) - u_2 p_2(t)] \log\left[\frac{p_1(t)w_2}{p_2(t)u_2}\right] \\
&\xrightarrow{\sigma t \gg 1} \frac{u_1 u_2 - w_1 w_2}{u_1 + u_2 + w_1 + w_2} \log\left[\frac{u_1 u_2}{w_1 w_2}\right] = (j_+ - j_-) \log\left(\frac{j_+}{j_-}\right) \geq 0
\end{aligned}
\tag{S51}
$$

$$
\begin{aligned}
\frac{\dot{h}_d(t)}{T} &= [w_1 p_2(t) - u_1 p_1(t)] \log\left[\frac{w_1}{u_1}\right] + [w_2 p_1(t) - u_2 p_2(t)] \log\left[\frac{w_2}{u_2}\right] \\
&\xrightarrow{\sigma t \gg 1} \frac{u_1 u_2 - w_1 w_2}{u_1 + u_2 + w_1 + w_2} \log\left[\frac{u_1 u_2}{w_1 w_2}\right] = (j_+ - j_-) \log\left(\frac{j_+}{j_-}\right) \geq 0
\end{aligned}
\tag{S52}
$$

$$
\begin{aligned}
\frac{\dot{f}_d(t)}{T} &= [w_1 p_2(t) - u_1 p_1(t)] \log\left[\frac{p_2(t)p_1^{ss}}{p_1(t)p_2^{ss}}\right] + [w_2 p_1(t) - u_2 p_2(t)] \log\left[\frac{p_1(t)p_2^{ss}}{p_2(t)p_1^{ss}}\right] \\
&= \sigma(p_2(t)p_1^{ss} - p_1(t)p_2^{ss}) \log\left[\frac{p_2(t)p_1^{ss}}{p_1(t)p_2^{ss}}\right] \geq 0 \\
&\xrightarrow{\sigma t \gg 1} 0
\end{aligned}
\tag{S53}
$$

The relaxation time to a steady state (NESS) from an arbitrary state in a far-from-equilibrium is $\sim \sigma^{-1} =$

$(u_1 + u_2 + w_1 + w_2)^{-1}$, and it is noteworthy that the entropy production inside the system $(T\dot{e}_p)$, the total heat production that will be discharged to the surrounding ($\dot{h}_d$), and the housekeeping heat ($\dot{Q}_{hk}$) are all identical at the steady state as $T\dot{e}_p = \dot{h}_d = \dot{Q}_{hk} \rightarrow (j_+ - j_-)\log(j_+/j_-) \geq 0$, and $\dot{f}_d = 0$. Here, $\dot{Q}_{ex}$, the residual of heat (excess heat, $\dot{Q}_{ex} = \dot{h}_d - \dot{Q}_{hk}$) for the nonequilibrium process, is zero at the steady state. Although obtained for 2-state model, the above expression, especially the total heat production (or housekeeping heat) at the steady state, $\dot{h}_d = T\dot{e}_p = \dot{Q}_{hk} = T(j_+ - j_-)\log j_+/j_-$ can easily be generalized for the N-state model.

5. RELATIONSHIP BETWEEN MOTOR DIFFUSIVITY AND HEAT DISSIPATION.

For $(N = 2)$-state model one can obtain an explicit expression that relates D with $\dot{Q}$ (for the case of $f = 0$) as follows. From the expressions of V (Eq.S7), D (Eq.S8), and $\dot{Q}$,

$$\dot{Q} = \frac{V}{d_0} k_B T \log \frac{u_1 u_2}{w_1 w_2} \qquad (S54)$$

Substitution of $u_1 = u_1(V)$ from Eq.S7 into Eq.S54 gives an expression of $\dot{Q}$ as a function of V:

$$\frac{\dot{Q}}{k_{cat} k_B T} = v \log \left[\frac{1 + \kappa v}{1 - v} \right]$$
$$= \sum_{n=1}^{\infty} \frac{1}{n} \left(1 + (-1)^{n-1} \kappa^n \right) v^{n+1} \qquad (S55)$$

where $v = V/V_{max}$ ($0 \leq v \leq 1$) and $\kappa \equiv \frac{k_{cat}(k_{cat} + w_1 + w_2)}{w_1 w_2}$. $\dot{Q}$ diverge as $v \rightarrow 1$; but for small $v \ll 1$, $\dot{Q}/(k_{cat} k_B T) \sim (\kappa + 1)v^2$, thus $v \sim \dot{Q}^{1/2}$.

As long as $\dot{Q}$ is small, one should expect from Eq.S9 that D increases with $\dot{Q}$ as

$$D = D_0 + \gamma_1 \dot{q}^{1/2} + \gamma_2 \dot{q} + \gamma_3 \dot{q}^{3/2} \qquad (S56)$$

where $\dot{q} \equiv \frac{\dot{Q}}{k_B T k_{cat}}$, $D_0 = \frac{d_0^2 k_{cat}}{\kappa + 1}$, $\gamma_1 = d_0^2 k_{cat} \frac{\kappa - 1}{2(\kappa + 1)^{3/2}}$, $\gamma_2 = -d_0^2 k_{cat} \frac{k_{cat}^2}{w_1 w_2}$, and $\gamma_3 = \frac{d_0^2 k_{cat}}{(\kappa + 1)^{5/2}} \frac{k_{cat}^2}{w_1 w_2}$.

For arbitrary number of states N, by using Eq.S12 and Eq.S15, $\dot{Q}$ can be written as

$$\dot{Q}/k_B T = \frac{V}{d_0} \log \frac{\prod_{i=1}^{N} u_i}{\prod_{i=1}^{N} w_i}$$
$$= \frac{V^{(D)}}{N} \log \left(1 + V^{(D)} \frac{\sum_{n=1}^{N} r_n \prod_{i=1}^{N} u_i}{N \prod_{i=1}^{N} w_i} \right)$$
$$= \frac{V^{(D)}}{N} \log \left(1 + \frac{V^{(D)}}{N} (A + B u_1) \frac{1}{C} \right)$$
$$= \frac{V^{(D)}}{N} \log \left(1 + \frac{V^{(D)}}{N} f(V^{(D)}/N) \right) \qquad (S57)$$

where we used $V^{(D)} = VN/d_0$, $f(V^{(D)}/N) = \frac{A}{C} + B \left(1 - B \frac{V^{(D)}}{N} \right)^{-1} \left(1 + \frac{A}{C} \frac{V^{(D)}}{N} \right)$, and Eqs.S12, S15, S16. The definitions of A, B, and C are identical to those in Eq.S15. Here $V^{(D)}/N$ corresponds to ATP hydrolysis rate. For $V^{(D)} \rightarrow 0$, $\dot{Q} \rightarrow 0$ is expected. Also for small $V^{(D)}$, $\dot{Q} \sim (\frac{A}{C} + B) \left(\frac{V^{(D)}}{N} \right)^2$. Thus, $V \sim \dot{Q}^{1/2}$. Since $D \sim V + \mathcal{O}(V^2)$ for small V, it follows that D can be written as a function of $\dot{q}$ as in the same form as Eq.S56.

6. RATE CONSTANTS, ENHANCEMENT OF DIFFUSION, AND CONVERSION EFFICIENCY DETERMINED FROM THE (N=2)-STATE KINETIC MODEL.

The values in the Table 1 were compiled based on the followings.

Catalase

In Ref.[9] $(\Delta D/D_0) = 0.28$ at $V = 1.7 \times 10^4 \ s^{-1}$; however, $V = 1.7 \times 10^4 \ s^{-1}$ is not the maximum catalytic rate. Because $\Delta D/D_0$ is approximately linear in V, the enhancement of diffusion at the maximal turnover rate $V_{max} = u_2 = 5.8 \times 10^4$ is estimated as $(\Delta D/D_0)_{obs} = 0.28 \times \frac{5.8 \times 10^4}{1.7 \times 10^4} = 0.96 \approx 1$.

Alkaline phosphatase

Similar to catalase, $(\Delta D/D_0)_{obs} = 0.77 \times \frac{1.4 \times 10^4}{5.5 \times 10^3} = 2.5 \approx 3$.

Estimate of $(\Delta D/D_0)_{max}$

Freely diffusing enzymes effectively perform no work on the surrounding environment; thus $-\Delta \mu_{eff} = Q$ with $W = 0$, which leads to $e^{Q/k_B T} = u_1 u_2/w_1 w_2$. By assuming that the substrate concentration $[S] \sim K_M = (u_2 + w_1)/u_1^o$, we get

$$e^{Q/k_B T} = \frac{u_1 u_2}{w_1 w_2} \sim \frac{u_1^o K_M u_2}{w_1 w_2} = \frac{(u_2 + w_1) u_2}{w_1 w_2} \geq \frac{u_2^2}{w_1 w_2} = \frac{k_{cat}^2}{w_1 w_2}.$$

This relation allows us to estimate the upper bound of $(\Delta D/D_0)_{max}$ as follows when $u_2 \gg w_1, w_2$ is satisfied.

$$\left(\frac{\Delta D}{D_0} \right)_{max} \approx \frac{k_{cat}^2}{2 w_1 w_2} \leq \frac{1}{2} e^{Q/k_B T}. \qquad (S58)$$

Alternatively, u_1 and w_2 of enzymes can be estimated by assuming (i) that the reaction is diffusion limited,

$u_1^o = 10^8 s^{-1} M^{-1}$, and (ii) that the substrate concentration $[S]$ is similar to Michaelis-Menten constant K_M ($[S] \sim K_M$). The two conditions $u_1 = u_1^o[S] \sim K_M \times 10^8$ (s^{-1}) and $K_M(= (u_2 + w_1)/u_1^o)$, and Q (heat measured by the calorimeter in ref. [9]), u_2, K_M which are available in ref. [9], provide all the rate constants including $w_1 = u_1^o K_M - u_2$ and $w_2 = \frac{u_1 u_2}{w_1 e^{Q/k_B T}}$, allowing us to calculate $\left(\frac{\Delta D}{D_0} \right)_{\max} = \frac{u_2^2 + (w_1 + w_2)u_2 - w_1 w_2}{2 w_1 w_2}$.

———————

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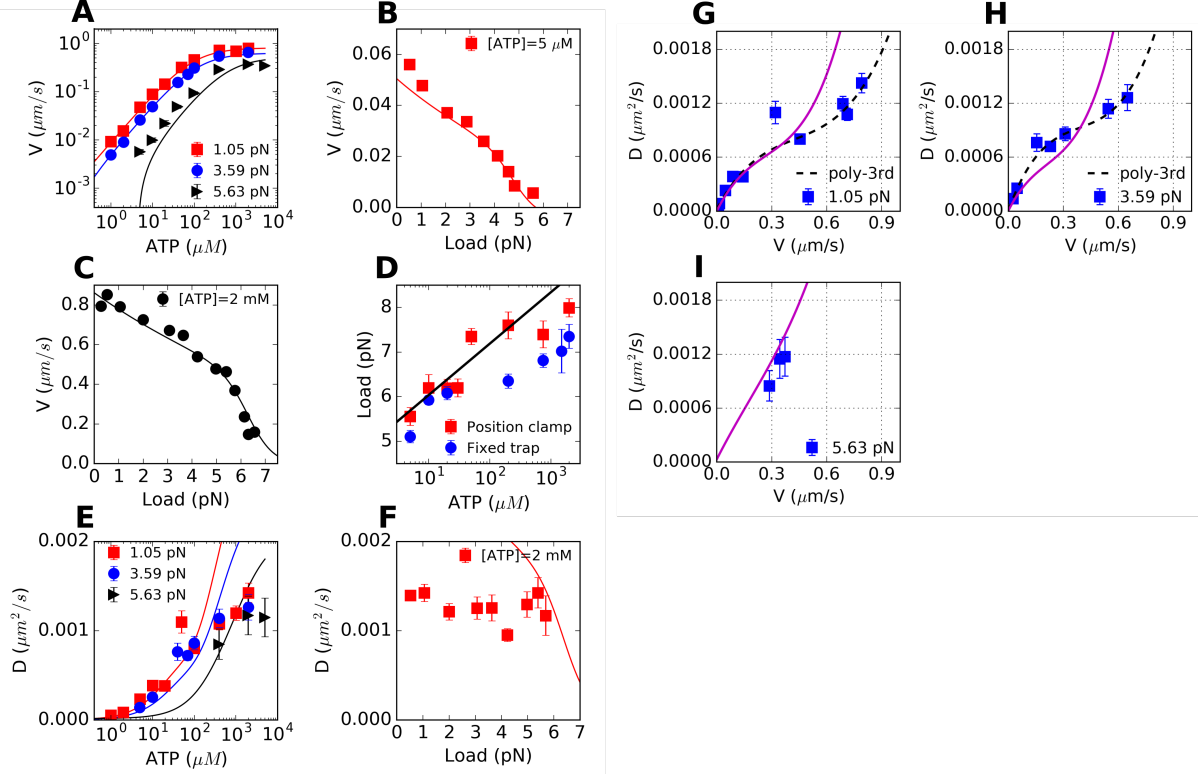[10] K. Visscher, M. J. Schnitzer, and S. M. Block, Nature **400**, 184 (1999).

FIG. S3. Analysis of experimental data, extracted from Ref. [10], but using (N=2)-state model. The solid lines are the fits to the data **A.** V vs ATP at f =1.05 pN (red square), 3.59 pN (blue circle), and 5.63 pN (black triangle). **B.** V vs load at [ATP] = 5 μM. **C.** V vs load at [ATP] = 2 mM. **D.** Stall force as a function of [ATP], measured by 'Position clamp' (red square) or 'Fixed trap' (blue circle) methods. **E.** D vs ATP at f =1.05 pN (red square), 3.59 pN (blue circle), and 5.63 pN (black triangle). D was estimated from $r = 2D/Vd_0$. **F.** D vs load at [ATP] = 2 mM. **G-I.** Motor diffusivity (D) as a function of mean velocity (V) for kinesin-1. (V,D) measured at varying [ATP] (= 0 – 2 mM) and a fixed (**G**) f =1.05 pN, (**H**) 3.59 pN, and (**I**) 5.63 pN [10]. The black dashed lines in **G** and **H** are the fits using Eq.5. The solid lines in magenta in **G-I** are plotted using the (N=2)-kinetic model.