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Supporting Material

Motor-substrate Interactions in Mycoplasma Motility Explains Non-Arrhenius Temperature Dependence

Jing Chen, John Neu, Makoto Miyata, and George Oster

SECTION I: DERIVATION OF PEEL-OFF RATE OF THE FOOT

As proposed in the main text, the peel-off process of the foot can be represented by the following Markov chain:

$$Q \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} Q-1 \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} \cdots \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} 1 \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} 0 \text{ (all-off)}$$

The average peel-off rate equals the reciprocal of the mean first passage time (MFPT) to reach state 0 (all-off), starting from state Q (all-on). In the following derivation we use the probability transition matrix to calculate the vector of MFPT starting from each state. The first component of the vector gives the peel-off rate. Suppose the system stays at state i at the present time. In time dT, the system jumps to state j with probability $r_{ij} \cdot dT$ (figure below), where r_{ij} is the transition rate from state i to state j.

In the peel-off model discussed here, the transition rates are

$$r_{ij} = \begin{cases} k_{on} & \text{if } j = i+1 \\ k_{off} & \text{if } j = i-1 \\ 0 & \text{otherwise} \end{cases}$$

Now the MFPT from state j is T_j, so the MFPT from state i is dT plus the sum of all T_j, weighted by the transition probability from i to j in dT.

The above reasoning is expressed as

$$T_i = \sum_{j \neq i} T_j r_{ij} dT + (1 - \sum_{j \neq i} r_{ij} dT) T_i + dT, \quad i = 1, \ldots, Q \tag{S1}$$

Rearranging the above equation and canceling the common factor dT yields

$$\sum_{j \neq i} T_j r_{ij} - T_i \sum_{j \neq i} r_{ij} = -1 \tag{S2}$$

Eq.S2 can be written in vector form as

$$\mathbf{P}^{\mathrm{T}} \mathbf{T} = \mathbf{-1} \tag{S3}$$

where $\mathbf{T} = \{T_i\}_{i=0,\ldots,Q}$ is the vector of MFPTs. Note that $T_0 \equiv 0$, since it takes no time to reach state 0 if the system starts from state 0. The operating matrix in Eq.S3 happens to be the transpose of the probability transition matrix of the Markov chain, $\mathbf{P}$.

$$
\mathbf{P} = \begin{bmatrix}
-k_{on} & k_{off} & & & & & \\
k_{on} & -k_{on}-k_{off} & k_{off} & & & & \\
& k_{on} & -k_{on}-k_{off} & & & & \\
& & k_{on} & \ddots & & & \\
& & & \ddots & & & \\
& & & \ddots & k_{off} & & \\
& & & & -k_{on}-k_{off} & k_{off} \\
& & & & k_{on} & -k_{off}
\end{bmatrix} \tag{S4}
$$

P is singular because all columns sum to zero. But $T_0 \equiv 0$ eliminates one unknown. Eq.S3 is solvable when the first column and the first row of **P** are removed and vector **T** is shortened by the first element. The solution to Eq.S3 gives the MFPT from state Q to state 0. Its reciprocal, the peel-off rate, is given in Eq.2 in the main text.

Solving Eq.S2 (i.e. Eq.S3 with the T_0 dimension removed) is shown in the following. There are altogether Q equations and Q unknowns:

$$
k_{off}T_{i-1} + k_{on}T_{i+1} - (k_{on} + k_{off})T_i = -1, \ \ i = 1,...,Q-1 \tag{S5}
$$

$$
k_{off}T_{Q-1} - k_{off}T_Q = -1 \tag{S6}
$$

Now let $\Delta T_i := T_{i+1} - T_i$, then Eq.S5 and Eq.S6 can be transformed into

$$
k_{on}\Delta T_i - k_{off}\Delta T_{i-1} = -1, \ \ i = 1,...,Q-1 \tag{S7}
$$

$$
-k_{off}\Delta T_{Q-1} = -1 \Rightarrow \Delta T_{Q-1} = 1/k_{off} \tag{S8}
$$

Let $\Delta T_i ' := \Delta T_i - \dfrac{1}{k_{off} - k_{on}}$, then Eq.S7 and Eq.S8 are equivalent to

$$
\Delta T_{i-1}' = K\Delta T_i' \tag{S9}
$$

$$
\Delta T_{Q-1}' = -\frac{K}{k_{off} - k_{on}} \tag{S10}
$$

where $K = k_{on}/k_{off}$.

Eq.S9 and Eq.S10 give

$$\Delta T_i' = K^{Q-i-1}\Delta T_{Q-1}' = -\frac{K^{Q-i}}{k_{off}-k_{on}} \tag{S11}$$

Thus,

$$\begin{aligned}
T_Q &= T_0 + \sum_{i=0}^{Q-1}\Delta T_i \\
&= \sum_{i=0}^{Q-1}\left(\Delta T_i' + \frac{1}{k_{off}-k_{on}}\right) \\
&= \frac{\sum_{i=0}^{Q-1}\left(1-K^{Q-i}\right)}{k_{off}-k_{on}} \\
&= \frac{1}{k_{off}(1-K)}\left(Q+1-\frac{1-K^{Q+1}}{1-K}\right) \\
&= \frac{1}{k_{off}}\frac{Q-(Q+1)K+K^{Q+1}}{(1-K)^2}
\end{aligned}$$

The reciprocal of the above gives the peel-off rate R_p:

$$R = k_{off}\,\frac{(1-K)^2}{Q-(Q+1)K+K^{Q+1}} \tag{S12}$$

SECTION II: DERIVATION OF WEAKLY-FACILITATED AND SPONTANEOUS RELEASE RATE OF THE FOOT

$$Q \underset{k_{on}'}{\overset{Qk_{off}'}{\rightleftharpoons}} Q-1 \underset{2k_{on}'}{\overset{(Q-1)k_{off}'}{\rightleftharpoons}} \cdots \underset{(Q-1)k_{on}'}{\overset{2k_{off}'}{\rightleftharpoons}} 1 \underset{Qk_{on}'}{\overset{k_{off}'}{\rightleftharpoons}} 0 \text{ (all-off)}$$

A Markov chain model similar to the one described above gives the weakly-facilitated release rate and the spontaneous release rate of the foot. In this model, we also have Q+1 states connected in a queue. But the transition rates between each pair of neighboring states change slightly, because the on/off event does not have to happen in a strictly sequential fashion. At state Q, any of the Q-i unbound sites can bind, and any of the i bound sites can unbind. The transition rates become

$$\begin{aligned}
r_{i-1\to i} &= (Q-i+1)\cdot k_{on} \\
r_{i\to i-1} &= i\cdot k_{off}
\end{aligned} \tag{S13}$$

and the transition matrix is

$$\mathbf{P} = \begin{bmatrix} -Qk_{on} & k_{off} & & & & & \\ Qk_{on} & -(Q-1)k_{on}-k_{off} & 2k_{off} & & & & \\ & (Q-1)k_{on} & -(Q-2)k_{on}-2k_{off} & & & & \\ & & (Q-2)k_{on} & \ddots & & & \\ & & & \ddots & & & \\ & & & \ddots & (Q-1)k_{off} & & \\ & & & & -k_{on}-(Q-1)k_{off} & Qk_{off} \\ & & & & k_{on} & -Qk_{off} \end{bmatrix} \quad \text{(S14)}$$

Because the foot can start from any state with corresponding residence probability, the reciprocal of the whole-foot rate is the weighted average MFPT. Solving Eq.S14 with similar procedures given in Section I gives Eq.4 in the main text.

The following is a simpler derivation of the same result. For each binding site the mean residence times of the unbound and the bound state are, $\tau_u = 1/k_{on}$, $\tau_b = 1/k_{off}$, respectively. The probability of finding a site in the unbound state is

$$p_u = \frac{\tau_u}{\tau_b + \tau_u} \quad \text{(S15)}$$

Suppose the Q states of the foot are categorized into two: the all-detached state $\{0\}$, and the compound state with at least one bound site $\{1,2,\ldots,Q\}$. The two newly defined states of the foot obey the same law as Eq.S15. The probability of the all-detached state is p_u^Q, as computed in Eq.S15. Let T_{off} be the mean residence time of the all-detached state, and T_{on} that of the compound state. Then we have

$$\frac{T_{off}}{T_{on} + T_{off}} = p_u^Q = \left(\frac{\tau_u}{\tau_b + \tau_u} \right)^Q \quad \text{(S16)}$$

T_{off} is the reciprocal of the rate of having any one of the Q sites bind to the substrate, which is Q times k_{on}. Substituting $T_{off} = 1/Qk_{on} = \tau_u/Q$ into Eq.S16 yields

$$T_{on} = \frac{\tau_u}{S}\left[\left(1 + \frac{\tau_b}{\tau_u} \right)^Q - 1 \right] \quad \text{(S17)}$$

Then the reciprocal of T_{on} is the foot unbinding rate, same as Eq.4 in the main text.

$$R = \frac{Q/\tau_u}{\left(1+\tau_b/\tau_u\right)^Q - 1} = \frac{Qk_{on}}{\left(1+k_{on}/k_{off}\right)^Q - 1} \tag{S18}$$

SECTION III: DERIVATION OF THE LOAD-VELOCITY CURVE

The following derivation takes into account of the weakly-facilitated foot release during the powerstroke and the spontaneous foot release during the re-stretching. The resultant load-velocity curve was shown in Figure 4*A* in the main text. Additional parameters and their values are listed in Table S1.

Consider the ensemble of feet which bind and unbind with the substrate (top left panel of Figure S1). Each foot is characterized by one continuous state variable, its displacement, x, relative to the beginning of a powerstroke, as seen in the cell's frame of reference. The important "checkpoints" are $x = 0$ (beginning of powerstroke), $x = \lambda$ (end of powerstroke) and $x = \lambda+L$ (unstressed backward position). When a foot completes a powerstroke crossing from $x < \lambda$ to $x > \lambda$, we assume that the motor hydrolyzes ATP, and is set to the "open" configuration.

In addition, we have three discrete states of the foot, one bound state and two unbound (thick horizontal bars, top left panel of Figure S1). The bound feet are stuck to the substrate, and in the cell's frame of reference, translate at the gliding velocity V. The ensemble density of bound feet is denoted $\rho_0(x)$, in $x \geq 0$. It is convenient to distinguish two states of unbound feet. Feet of the first state has unbounded during the powerstroke ($0 < x < \lambda$). They are rapidly pulled to $x = \lambda$ at velocity f_m/ζ_f, where f_m is the motor force, and ζ_f the hydrodynamic drag coefficient of the foot. The ensemble density of these feet is denoted $\rho_1(x)$, in $0 \leq x \leq \lambda$. The second unbound state accounts for the returning feet heading back to $x = 0$ at velocity $-f_r/\zeta_f$. Here, $-f_r$ is the weak restoring force that drives the kinking of the leg and returning of the feet. The density of the returning feet is denoted $\rho_2(x)$, in $x \geq 0$.

Now we write the steady state (time independent) transport equations for $\rho_0(x)$, $\rho_1(x)$ and $\rho_2(x)$. $\rho_0(x)$ satisfies the ODE:

$$V\frac{d\rho_0}{dx} = -R(x)\rho_0 \tag{S19}$$

The LHS of Eq.S19 is the convective derivative (time derivative of $\rho_0(x(t))$, at $x(t)$ with $\dot{x} = V$). $R(x)$ is the rate coefficient for foot unbinding. We expect a piecewise character in $R(x)$:

$$R(x) = \begin{cases} R_{wf}(x), & 0 \leq x < \lambda \\ R_s(x), & \lambda \leq x < \lambda + L \\ R_p(x), & x \geq \lambda + L \end{cases} \tag{S20}$$

Here, R_{wf} denotes the weakly facilitated release during the powerstroke, R_s the spontaneous release rate and R_p the peel-off rate. According to the amount of force acting on the foot in each case, the relative magnitude of the three rates should be $R_p > R_{wf} > R_s$.

The transport ODE for $\rho_1(x)$ in $0 \le x \le \lambda$ is based on translational velocity $\dot{x} = f_m/\zeta_f$, and the source due to foot release in $0 < x < \lambda$.

$$\frac{f_m}{\zeta_f}\frac{d\rho_1}{dx} = R(x)\rho_0, 0 \le x \le \lambda \tag{S21}$$

Since all feet at the beginning of the powerstroke are assumed to be bound, we have the boundary condition:

$$\rho_1(0) = 0 \tag{S22}$$

The transport equation for $\rho_2(x)$, the returning foot, is based on the translational velocity $\dot{x} = -f_r/\zeta_f$ and the sources indicating the spontaneous foot release and the peel-off:

$$-\frac{f_r}{\zeta_f}\frac{d\rho_2}{dx} = \begin{cases} 0, & 0 \le x < \lambda \\ R(x)\rho_0, & x \ge \lambda \end{cases} \tag{S23}$$

Finally, we have two flux balance boundary conditions:

$$V\rho_0(0) = \frac{f_r}{\zeta_f}\rho_2(0) \tag{S24}$$

$$V\left(\rho_0(\lambda^+) - \rho_0(\lambda^-)\right) = \frac{f_m}{\zeta_f}\rho_1(\lambda) \tag{S25}$$

The LHS of Eq.S24 is the flux of the unbound feet returning to $x = 0$, and the RHS the flux of feet starting the powerstroke. The balance holds upon the assumption that the powerstroke starts as soon as a foot returns to $x = 0$. Eq.S25 represents the jump of foot density at $x = \lambda$ contributed by the rebinding of the foot that have unbound during the powerstroke. This equation holds when we assume that the rebinding happens very fast compared to the time scales resolved in these equations.

Eqs.S19-S25 determines $\rho_0(x)$, $\rho_1(x)$ and $\rho_2(x)$ up to a multiplicative constant. This constant can be determined by normalization:

$$\int_0^\infty \rho_0(x)dx + \int_0^\lambda \rho_1(x)dx + \int_0^\infty \rho_2(x)dx = 1 \tag{S26}$$

Assume all foot release rates are invariant with position. Then Eqs.S19-S26 can be solved analytically with solutions:

$$TV\cdot\rho_0(x)=\begin{cases}\exp\left(-\dfrac{R_{wf}x}{V}\right), & 0\le x<\lambda\\ \exp\left(-\dfrac{R_s(x-\lambda)}{V}\right), & \lambda\le x<\lambda+L\\ \exp\left(-\dfrac{R_sL+R_p(x-\lambda-L)}{V}\right), & x\ge\lambda+L\end{cases}$$

$$TV\cdot\rho_1(x)=\frac{\zeta_f V}{f_m}\left(1-\exp\left(-\frac{R_{wf}x}{V}\right)\right), \qquad 0\le x\le\lambda$$

$$TV\cdot\rho_2(x)=\begin{cases}\dfrac{\zeta_f V}{f_r}, & 0\le x<\lambda\\ \dfrac{\zeta_f V}{f_r}\exp\left(-\dfrac{R_s(x-\lambda)}{V}\right), & \lambda\le x<\lambda+L\\ \dfrac{\zeta_f V}{f_r}\exp\left(-\dfrac{R_sL+R_p(x-\lambda-L)}{V}\right), & x\ge\lambda+L\end{cases} \tag{S27}$$

where T is the average duration of the whole cycle.

$$\begin{aligned}T=&\frac{1-\exp\left(-R_{wf}\lambda/V\right)}{R_{wf}}+\frac{1-\exp\left(-R_sL/V\right)}{R_s}+\frac{\exp\left(-R_sL/V\right)}{R_p}\\&+\frac{\zeta_f}{f_m}\left(\lambda-V\frac{1-\exp\left(-R_{wf}\lambda/V\right)}{R_{wf}}\right)\\&+\frac{\zeta_f}{f_r}\left(\lambda+V\frac{1-\exp\left(-R_sL/V\right)}{R_s}+V\frac{\exp\left(-R_sL/V\right)}{R_p}\right)\end{aligned} \tag{S28}$$

The first three terms in Eq.S28 correspond to the average time the bound foot spends during the powerstroke, re-stretching and peel-off respectively. The 4th term represents the time it takes for the free foot to reach the post-power-stroke position after its weakly facilitated release from the substrate. The last term represents the time that the free foot resets to the front position after either the spontaneous release or the peel-off. The load-velocity relation is calculated with the force balance equation. The load force is balanced by the net force contributed by all feet. The bound feet provide positive force during the powerstroke, negative force during the peel-off process, and the weak resetting force during the re-stretching. The free foot in state 1 is dragged in the medium with the motor force; and the free foot in state 2 is dragged with the resetting force. Therefore, the force balance equation reads

$$\left(F_L+\zeta_b V\right)/N = f_m\int_0^{\lambda}\rho_0(x)dx - f_r\int_{\lambda}^{\lambda+L}\rho_0(x)dx - \int_{\lambda+L}^{\infty}\kappa(x-\lambda-L)\rho_0(x)dx$$
$$+f_m\int_0^{\lambda}\rho_1(x)dx - f_r\int_0^{\infty}\rho_2(x)dx \tag{S29}$$

Plugging in Eq.S27 gives

$$F_L = -\zeta_b V + \frac{N}{T}\left\{\frac{f_m-\zeta_f V}{R_{wf}}\left(1-\exp\left(-\frac{R_{wf}\lambda}{V}\right)\right) - \frac{\left(\kappa/R_p-\zeta_f\right)V}{R_p}\exp\left(-\frac{R_s L}{V}\right) + \frac{f_r-\zeta_f V}{R_s}\left(1-\exp\left(-\frac{R_s L}{V}\right)\right)\right\} \tag{S30}$$

The impulse balance equations given in the main text (Eq.1 and Eq.3) are simplified version of Eq.S30. Eq.3 corresponds to the case where ζ_f, ζ_b, R_s, $f_r \rightarrow 0$. These assumptions have been elaborated in the main text before the introduction of the impulse balance equations. Eq.1 is the further simplification when $R_{wf} << V/\lambda$.

The derivation of the $V < 0$ case is similar, as illustrated by the top right panels of Figure S1. There are again three states of the foot, albeit with different meanings for the states of the free foot. This is because the leg cycle in the negative regime is asymmetric to that in the positive regime. The major break-off of the foot from the substrate occurs during the powerstroke when the leg is overstretched. Since the force is exerted in the opposite direction of the tip of the foot, it does not create a peel effect. The break-off process is similar to the weakly-facilitated and spontaneous release, only with much stronger force facilitation. Therefore, we labeled the new rate as R_{sf} to stand for "strongly-facilitated". The leg will be over-relaxed after the motor releases ADP and opens up. It re-stretches while the foot moves on towards the starting position for the next powerstroke. During the re-stretching the foot can also spontaneously release from the substrate with essentially the same rate used in the case $V > 0$. Now ρ_1 represents the density of the foot that has been spontaneously released during re-stretching. ρ_2 corresponds to the foot that has been snatched off the substrate during the powerstroke. The governing transport ODEs are given in Eqs.S31.

$$
\begin{cases}
V\dfrac{d\rho_0}{dx} = -R(x)\rho_0, & & \text{(a)} \\
-\dfrac{f_r}{\zeta_f}\dfrac{d\rho_1}{dx} = R(x)\rho_0, & 0 < x \le \lambda & \text{(b)} \\
\dfrac{f_m}{\zeta_f}\dfrac{d\rho_2}{dx} = \begin{cases} R(x)\rho_0, & x \le 0 \\ 0, & 0 < x \le \lambda \end{cases} & & \text{(c)} \\
-V\rho_0(\lambda) = \dfrac{f_m}{\zeta_f}\rho_2(\lambda), & & \text{(d)} \\
-V\left(\rho_0(0^-) - \rho_0(0^+)\right) = \dfrac{f_r}{\zeta_f}\rho_1(0), & & \text{(e)} \\
\rho_1(\lambda) = 0, & & \text{(f)} \\
\int_{-\infty}^{\lambda}\rho_0 dx + \int_0^{\lambda}\rho_1 dx + \int_{-\infty}^{\lambda}\rho_2 dx = 1, & & \text{(g)}
\end{cases}
\tag{S31}
$$

with the piecewise foot release rate

$$
R(x) = \begin{cases} R_{sf}(x), & x \le 0 \\ R_s(x), & 0 < x \le \lambda \end{cases} \tag{S32}
$$

The meanings of each equation above are similar to those for positive velocities. The resulting load force as a function of velocity is

$$
F_L = -\zeta_b V + \frac{N}{T}\left\{\frac{f_m - \zeta_f V}{R_{sf}} - \frac{\kappa V}{R_{sf}^2} - \frac{f_r + \zeta_f V}{R_s}\left(1 - \exp\left(\frac{R_s\lambda}{V}\right)\right)\right\} \tag{S33}
$$

where the average cycle duration is

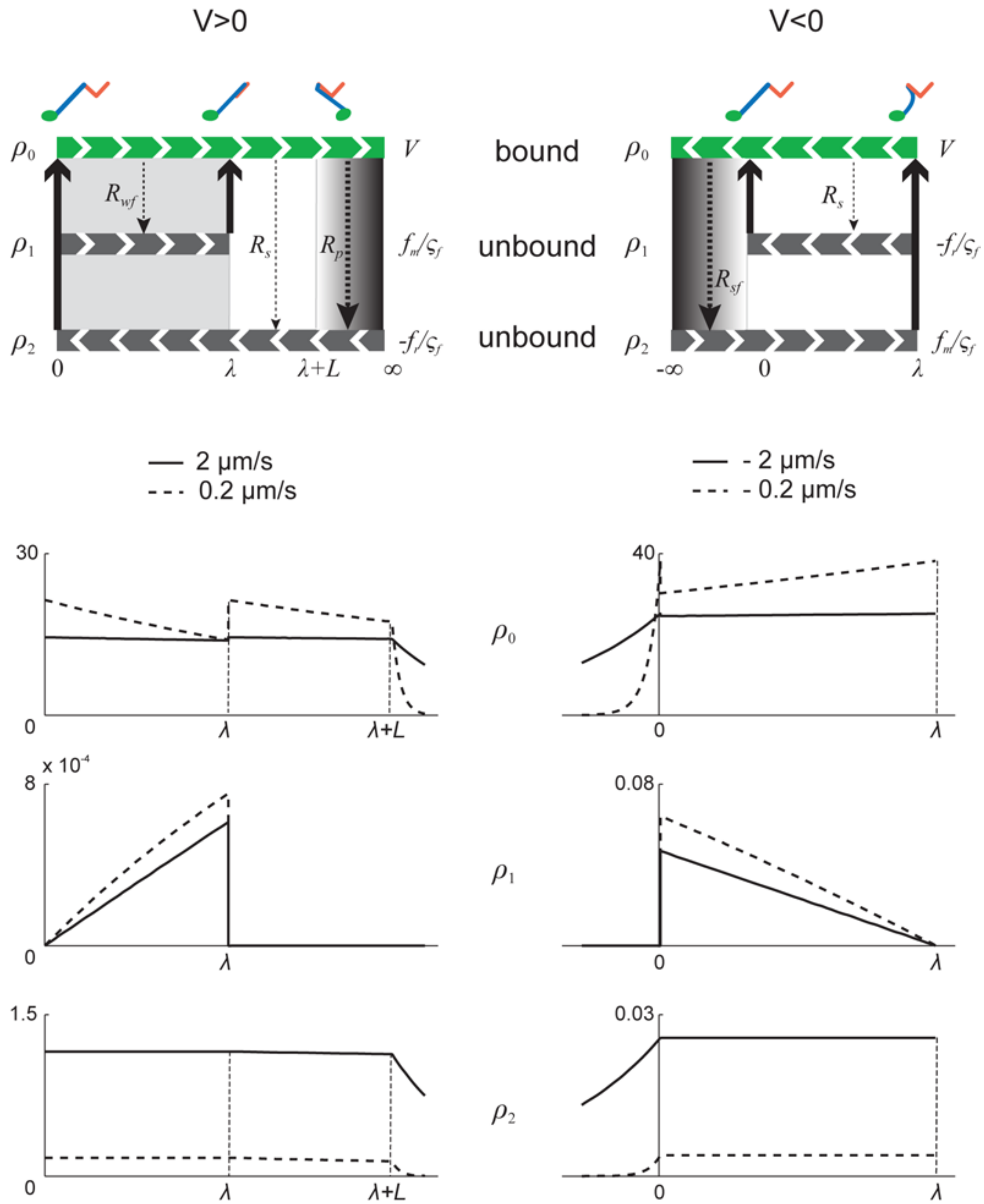
$$
T = \frac{1}{R_{sf}} + \frac{1 - \exp\left(R_s\lambda/V\right)}{R_s} + \frac{\zeta_f}{f_m}\left(\lambda - \frac{V}{R_{sf}}\right) + \frac{\zeta_f}{f_r}\left(\lambda + V\frac{1 - \exp\left(R_s\lambda/V\right)}{R_s}\right) \tag{S34}
$$

Similar to Eq.S28, the first two terms in Eq.S34 correspond to the average time the foot spends during the powerstroke and the re-stretching. The other two represent the average resetting time after the foot releases from the over-stretched position and the unstretched position. The duty ratio equals the sum of the first two terms divided by the cycle period.

Typical foot density distributions of each state in the $V > 0$ and $V < 0$ cases are shown in Figure S1. ρ_0 dominates over ρ_1 and ρ_2 in magnitude in both $V > 0$ and $V < 0$ cases because the unbound foot translocates very fast with a small hydrodynamic drag coefficient, leading to a small residence time. Furthermore, the magnitude of the density of each unbound state is determined by the corresponding driving force during the state and the foot release rate. For example, ρ_1 at $V > 0$ is extremely small because the foot is released with the weakly facilitated rate R_{wf}, and then driven fast by the relatively large force f_m. By contrast, ρ_2 at $V > 0$ is much larger because the majority of the foot is released by the much larger peel-off rate R_p, and then driven by the much smaller restoring force f_r.

Table S1: List of additional parameters used in the computation of the load-velocity curve.

Parameters	***Value***	***Physical meaning***
L	25 nm	distance between the end of powerstroke and backward re-stressed position
f_r	0.005 pN	weak resetting force
ζ_f	200 pN·s/m	hydrodynamic drag coefficient of the foot
ζ_b	2×10^4 pN·s/m	hydrodynamic drag coefficient of the cell body
k''_{off}	1.7×10^3 s^{-1} (16.8 k_BT)	spontaneous release rate of single site (and its Arrhenius factor)



V>0
V<0
bound
unbound
unbound
ρ0
ρ1
ρ2
V
Rwf
Rs
Rp
Rsf
fm/ςf
-fr/ςf
0
λ
λ+L
∞
-∞
2 μm/s
0.2 μm/s
- 2 μm/s
- 0.2 μm/s
30
40
x 10-4
8
0.08
1.5
0.03

Figure S1: Illustration of the transport equations and the resultant density distribution of the feet. The cartoons on the top illustrate Eqs.S19-S26 and Eqs.S31. Left: V > 0. The horizontal bars show the three different states of the foot, bound (ρ_0), released during the powerstroke with weak facilitation (ρ_1), and spontaneously released or peeled off after the powerstroke (ρ_2). Corresponding foot conformations are labeled on the very top. The directions of foot transport in these states are shown with white arrows in the bar and velocities labeled on the right end, both in the frame of reference of the cell body. The thick solid arrows pointing upward illustrate the rebinding of the free foot. The dashed arrows pointing downward show different ways that the foot can release from the substrate, their thickness indicating the relative magnitude of the rates. The shadings illustrate the forces acting on the foot: motor force during the powerstroke (*light even shade*) and peel force after stretched (*monotonically darker shade*). Right: V < 0. All labels bear similar meanings. There are also three different states of the foot, bound (ρ_0), spontaneously released during the post-power-stroke relaxation (ρ_1), and snatched off during the powerstroke (ρ_2). The diagrams below the cartoons show examples of typical density distribution of the feet at different states at 22.5ºC.