Motor-Substrate Interactions in *Mycoplasma* Motility Explains Non-Arrhenius Temperature Dependence

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ABSTRACT Mycoplasmas exhibit a novel, substrate-dependent gliding motility that is driven by ~400 “leg” proteins. The legs interact with the substrate and transmit the forces generated by an assembly of ATPase motors. The velocity of the cell increases linearly by nearly 10-fold over a narrow temperature range of 10–40°C. This corresponds to an Arrhenius factor that decreases from ~45 $k_B T$ at 10°C to ~10 $k_B T$ at 40°C. On the other hand, load-velocity curves at different temperatures extrapolate to nearly the same stall force, suggesting a temperature-insensitive force-generation mechanism near stall. In this article, we propose a leg-substrate interaction mechanism that explains the intriguing temperature sensitivity of this motility. The large Arrhenius factor at low temperature comes about from the addition of many smaller energy barriers arising from many substrate-binding sites at the distal end of the leg protein. The Arrhenius dependence attenuates at high temperature due to two factors: 1), the reduced effective multiplicity of energy barriers intrinsic to the multiple-site binding mechanism; and 2), the temperature-sensitive weakly facilitated leg release that curtails the power stroke. The model suggests an explanation for the similar steep, sub-Arrhenius temperature-velocity curves observed in many molecular motors, such as kinesin and myosin, wherein the temperature behavior is dominated not by the catalytic biochemistry, but by the motor-substrate interaction.

INTRODUCTION

Mycoplasmas are a genus of wall-less bacteria with compact genomes that may have arisen as a result of retrograde evolution (1). They are the smallest known free-living, self-replicating organisms. Despite the loss of many biological functions, mycoplasmas demonstrate a novel gliding motility on solid substrates, such as glass, plastic, and surface of epithelial cells (2–4). Their locomotion is always in the direction of a characteristic membrane protrusion at one pole of the cell (the “nose”) (5–8). The mechanism of this motility is novel since the *Mycoplasma* genome contains no homologs to genes associated with known mechanisms of bacterial motility (9–12).

The motility studies are carried out mainly on the fastest gliding species, *Mycoplasma mobile*. Under lab conditions, *M. mobile* glides smoothly and continuously on glass surface with velocities of 2.0–4.5 μm/s, or 3–7 body lengths/s (13). The energy source is ATP hydrolysis (14–16). Recent experiments reveal a complicated motility organelle in its nose (17). The core of the organelle consists of a dock structure fixed at the distal end of the nose, and dozens of filaments extending radially from the dock. These filaments anchor ~400 single protein “legs” that protrude through the cell membrane and interact with the substrate (Fig. 1) (18–22). Since the leg is the best studied protein in the complicated organelle, our model focuses on how these legs harness the forces generated by the ATPase motors to drive the motion of the cell.

*M. mobile* shows intriguing velocity changes with temperature and load force. The velocity increases almost linearly by ~10-fold over a narrow temperature range from 10°C to 40°C (see Fig. 3 A) (23). Translated onto a $1/T \sim \log V$ plot (see circles in Fig. 3 B), these data correspond to an Arrhenius factor that decreases from ~45 $k_B T$ at 10°C to ~10 $k_B T$ at 40°C. On the other hand, the velocity decreases nearly linearly with increasing load force, but the stall force extrapolates to ~25 pN at different temperatures (compare to Fig. 4 in Miyata et al. (23)). Cells attached to micro-beads trapped by optical tweezers also stall when pulled by a force of ~25 pN (23). These data suggest that the force-generation step is insensitive to temperature near stall loads.

In this article, we propose a leg-substrate interaction mechanism to explain the non-Arrhenius temperature dependence of *Mycoplasma* motility. In this mechanism, the release of the leg from the substrate is the major temperature-sensitive factor. Soo and Theriot (24) suggested in their model for *Listeria* motility that the large Arrhenius factor for the cell velocity is caused by the cooperative break-off of multiple binding sites so that the Arrhenius factors of single sites add. Our model goes further and explains the decrease in the Arrhenius factor as temperature rises, i.e., the sub-Arrhenius relationship between temperature and velocity. The model can be generalized to explain similar temperature sensitivity observed in many “walking” molecular motors such as kinesin and myosin (25,26). This theory reveals the motor-substrate interaction, especially the unbinding process, as the dominant factor affected by temperature, albeit not in a simple Arrhenius fashion.
MODEL AND RESULTS

In the following sections, we first lay down the framework for the motility process and the basic assumptions used in our model. After that, we go into the details of the leg-substrate interaction. In particular, we show that the multiple substrate-binding sites on the leg contribute to the steep, sub-Arrhenius temperature-velocity curve. In addition, we rectify the remaining deviation of the results at high temperatures by the weakly facilitated foot release during the power stroke. Finally, we show that the resultant load-velocity curve fits with the experimental data and explains the dynamical trajectories observed in optically trapped Mycoplasma cells, as well as the temperature-insensitive stall force.

Leg cycle

The sequence and geometric shape of the leg protein in Mycoplasma has been deduced from electron microscopy studies. The protein looks like a music note (Fig. 1; see also Fig. 9 in Adan-Kubo et al. (21)). The two short arms at the proximal end assume an open or a closed conformation, suggesting that the opening and closing motion is driven by the ATPase motor (16). The distal end bulges into a “foot” that interacts with the negatively charged substrate through multiple basic amino acids. The proximal arms and the foot are connected by a long segment. Atomic-force microscopy experiments suggest that this long segment is quite flexible (27), so that its mechanical property resembles that of a rope, i.e., exerting much less resistance to being compressed than being stretched.

Based on the structure of the leg protein and the proposed motility mechanism in Miyata (28), we modeled the mechanochemical cycle of a single leg as shown in Fig. 2 A.

The mechanochemical cycle begins with the leg in the front position and the foot bound to the substrate. When ATP loads into the motor, the motor carries out a power stroke and pulls on the foot. This process exerts a forward force on the cell body. After the power stroke, the cell continues moving forward, driven by the collective work of the other legs. The foot lags behind. The long segment becomes slack and exerts no force until the foot reaches the backward position and restretches the long segment. The long segment pulls the foot off the substrate. Then the leg resets to the front position, the foot rebinds to the substrate, and the cycle repeats.

Assumption 1. The hinge connecting the proximal arm and the long segment is weakly elastic, with its rest state in the front position. This provides the resetting force for the leg.

Assumption 2. The motor can bind ATP only after the leg fully resets to the front position. It can be explained by hidden coordinates for the motor (see Appendix). This assumption, together with Assumption 3, ensures that the power stroke always starts from the front position. This is for analytical convenience, and does not change the essential features of the model.

Assumption 3. The foot only rebinds to the substrate after it fully resets to the front. We picture the long segment behaving like a Venetian blind: the long segment kinks easily under a backward force and the kink propagates down toward the foot while the segment resets to the front position (leftmost panel of Fig. 2 A). During resetting, the kink keeps the foot in an unfavorable angle to the substrate, preventing its binding until the resetting completes. During

FIGURE 1 Motility apparatus of M. mobile. Four-hundred leg proteins are located at the neck of the M. mobile cell. Each leg assumes a music-note-like shape (zoom-in view), with two arms at the proximal end, and a long flexible segment (blue) with a foot (green) that interacts with the substrate.
impulse balance: the latter is equivalent to the balance of force impulses. Times ensures the balance of the ensemble average force; and steps, the only force is the weak elastic resetting force, which imposes a negative force. During the restretching and resetting and the foot tethered beyond the backward restressed position, applies a negative force (blue arrows). (B) Mechanism of foot peeling. The foot interacts with the sialic acids in the substrate through multiple binding sites. The bonds are shown by green projections in the zoom-in view on the right. When the stretched intermediate segment pulls on the foot from one end, most of the tension is exerted on the frontmost bond and thus significantly facilitates its unbinding, analogous to peeling-off a Velcro strip. In the idealized case, the bonds break off sequentially, forming a Markov process as shown in the sequence of events on the right. The Markov process gives an average peel-off rate of the foot as in Eq. 2.

Assumption 4. The foot releases more easily when it is pulled forward after the power stroke (step 3 in Fig. 2 A). This is because the long segment is attached to the posterior end of the foot so that it imposes a peeling force when it pulls the foot forward, as shown in Fig. 2 B. This mechanical asymmetry is necessary for net forward motion.

The velocity of the cell is computed from the force balance on the legs. Since inertial forces are negligible at such low Reynolds number, the load force and hydrodynamic-drag force on the cell body is equal to the total force generated by the motility organelle. The estimated hydrodynamic drag on the micron-sized cell body moving with a velocity of \(-1\) \(\mu\)m/s is \(-10^{-2}\) pN, much smaller than the scale of the external load force applied in the experiments; therefore, it can be neglected. The motility organelle generates force mainly by two steps in the leg cycle: the power stroke generates a positive force and the foot tethered beyond the backward restressed position imposes a negative force. During the restretching and resetting steps, the only force is the weak elastic resetting force, which we treat as negligibly small. Intuitively, the force balance at all times ensures the balance of the ensemble average force; and the latter is equivalent to the balance of force impulses. Thus, the force balance can be conveniently expressed as an impulse balance:

\[
\text{Load force } \times \text{ cycle period} = \# \text{ legs} \\
\times (\text{impulse from power stroke} \\
- \text{impulse from tethered foot}).
\]

In Section III of the Supporting Material, we derive the full version of the force balance equation from the transport equations for the density of feet. These equations can be reduced to the above equation when we neglect the hydrodynamic drag forces and consider the high velocity case.

At zero load, the two impulses in the parentheses cancel, leading to Eq. 1. Here \(V\) is the velocity of the cell, \(f_m\) is the motor force, \(\lambda\) is the power stroke length, \(\kappa\) is the elastic constant of the intermediate segment, and \(R_p\) is the peel-off rate of the foot. The computation of \(R_p\) leads to the most important conclusion of this article and will be discussed in detail in the following section. On the left-hand side of Eq. 1, \(\lambda/V\) is the mean residence time of the foot in the power stroke. Therefore, the mean impulse delivered by a single foot in one power stroke is \(f_m\lambda/V\). On the right-hand side, \(R_p\) is the average stretching of foot from the backward restressed position, and thus, \(\kappa V/R_p\) is the average force acting on the backward foot. Since the lifetime of backward bound state is \(1/R_p\), the impulse delivered per foot in this part of the cycle is \((\kappa V/R_p) \times (1/R_p) = \kappa V R_p^2\).

\[
f_m \lambda \frac{\lambda}{V} = \frac{\kappa V}{R_p^2} \Rightarrow V = \sqrt{\frac{f_m \lambda}{\kappa R_p}}.
\]

Equation 1 shows that the unloaded velocity is proportional to the peel-off rate of the foot. The temperature dependence of the velocity follows that of the peel-off rate, as we show in the following that terms under the square-root are approximated temperature-invariant. The power stroke length, \(\lambda\), is determined by the geometry of the motor and the leg, and should not change significantly with temperature. The motor force depends on temperature approximately linearly, i.e., \(f_m = \Delta G/\lambda = (\Delta H - T \Delta S)/\lambda\); it changes by \(-10\%\) over the temperature range of 10°C – 40°C, far from enough to account for the 10-fold increase in velocity. The elastic constant of the stretched leg, \(\kappa\), depends on the configuration of the intermediate segment. Using the same argument for the motor force, the elastic constant resulting from the
entropic part of the spring is a linear function of temperature (29), and does not change much in the relevant temperature range. The enthalpic part of the spring is usually attributed to chemical bonds. Since the enthalpy of a chemical bond is generally insensitive to temperature, so is the resultant spring constant.

In the next section we will derive the temperature dependence of the foot peel-off rate with an embedded submodel of the foot-substrate interaction. The submodel explains the steep, sub-Arrhenius temperature-velocity curve, except for some deviation in the high temperature regime.

Foot-substrate interaction

We now consider the foot-substrate interaction in more detail. This is the core part of the model, which explains the steep, sub-Arrhenius temperature-velocity curve.

The foot anchors to the negatively charged sialic acids in the substrate (30). The C-terminal domain of the leg protein, which constitutes the bottom part of the foot, contains multiple positively charged amino acids (18 Arg, 21 Lys). It is likely that specific binding sites for sialic acid form around these basic amino acids. Previous studies on the sialoadhesin receptor shows that the sialic acid binding site consists of two key amino acids with positive charges (31). This gives an estimate of <20 binding sites on the foot of Mycoplasma, based on which we used 10 in our model. That is, the foot is modeled as an anchoring strip with 10 sites that holds on to the substrate.

The asymmetric geometry of the leg protein suggests that the foot releases from the substrate more easily when pulled forward instead of backward. A backward pulling force, as that during the power stroke, is distributed almost equally among all the binding sites. A forward force, however, is concentrated mostly on the rearmost site, largely facilitating its unbinding. After the rearmost site unbinds, the next one undertakes most of the external force and unbinds quickly; and it goes on until all sites unbind. This process is analogous to peeling off a Velcro strip from one end to the other—by contrast it is much harder to rip off the Velcro by exerting an evenly distributed force on it.

The peeling-off of the foot can be modeled by a Markov process as shown in Fig. 2 B. Since the rearmost binding site is much more likely to unbind, the unbinding of different sites takes place approximately sequentially. Let \( Q \) be the number of binding sites. The corresponding Markov process consists of \( Q+1 \) states, each indicating the order of the current rearmost bound site, plus the “all-off” state:

\[
Q \xrightarrow{k_{on}} Q - 1 \xrightarrow{k_{on}} \cdots \xrightarrow{k_{on}} 1 \xrightarrow{k_{off}} 0 \text{ (all-off)}. 
\]

If the on- and off-rates of all binding sites are identical, the derivation presented in Section I of the Supporting Material gives the peel-off rate of the whole foot as

\[
R_p = k_{off} \times \frac{(1 - K)^2}{Q - (Q + 1)K + K^{Q+1}}, \tag{2}
\]

where \( K = k_{on}/k_{off} \) is the binding constant of a single site. As temperature increases, the enhanced thermal fluctuations facilitates the unbinding, thus decreasing the binding constant, \( K \). Equation 2 satisfies the following properties:

Low temperature limit : \( \Rightarrow R_p \rightarrow k_{off}/Q \).

High temperature limit : \( \Rightarrow R_p \rightarrow k_{off}/K^{Q-1} \).

If the binding constant, \( K \), depends on temperature in an Arrhenius way, then in the low temperature limit, the Arrhenius factor of the foot peel-off rate, \( R_p \), is approximately the Arrhenius factor of \( K \) multiplied by the number of sites. This multiplicity effect, however, attenuates as temperature increases; eventually, the effective Arrhenius factor tends to approximately the Arrhenius factor of \( K \) at the high temperature limit.

The feature of the model discussed so far leads to the steep, yet sub-Arrhenius temperature dependence of the velocity (dashed line in Fig. 3 A). Data fitting gives the values of the single site rates, \( k_{on} \) and \( k_{off} \), as listed in Table 1. Fig. 3 B compares the Arrhenius plot of the single-site unbinding rate, the whole-foot release rate, and the temperature-velocity data. Each site bears a factor of 10 \( k_B T \) (Table 1). However, the Arrhenius factor of the whole-foot rate amounts to \(-45 k_B T \) at 10\(^\circ\)C, and attenuates to \(-10 k_B T \) at 40\(^\circ\)C.

Weakly facilitated foot release during the power stroke rectifies the high-temperature curve

During the power stroke, the foot may also release from substrate. This foot release rate is much smaller than the peel-off rate at low temperature, but becomes significant as temperature increases. In this case, all the binding sites share the burden of the motor force. With much weaker facilitation than in the peel-off case, the energy barrier to break the binding of a site remains high and thus the unbinding rate bears a much larger Arrhenius factor. As a consequence, the overall foot release rate increases acutely with temperature (Fig. 3 C).

The foot release curtails the power stroke, and consequently reduces the velocity. This is shown by the velocity dependence in Eq. 3. Here \( R_{wf} \) denotes the weakly facilitated foot release rate:

\[
f_m \frac{1 - \exp\left(-R_{wf} \lambda/V\right)}{R_{wf}} = \frac{\kappa V}{R_p^2} \Rightarrow V = \frac{1 - \exp\left(-R_{wf} \lambda/V\right) f_m R_p^2}{R_{wf} \kappa}. \tag{3}
\]

The fractional term on the left-hand side of Eq. 3 stands for the average duration of the effective power stroke. It is computed from
The weakly facilitated foot release also corresponds to a Markov process. The Markov states stand for the number of binding sites currently bound. The binding sites do not have to unbind in a certain order, so the forward rate from state $i$ to $i-1$ equals $k_{\text{on}}$ to account for the fact that every bound site has an equal chance to unbind. Similarly the backward rate from state $i$ to $i+1$ equals $(Q-i)k_{\text{off}}$.

$$Q = \frac{Q_{\text{on}}}{k_{\text{on}}^2} = \frac{2k_{\text{off}}}{2k_{\text{on}} + 2k_{\text{off}}} = \frac{k_{\text{on}}}{k_{\text{off}}} = 1 \quad \left( Q = 1 \text{ (all-off)} \right).$$

With the derivation given in Section II of the Supporting Material, we obtain the weakly facilitated foot release rate as Eq. 4. It is computed with $k_{\text{on}}'$ and $k_{\text{off}}'$ given in Table 1,

$$R_{\text{wf}} = \frac{Qk_{\text{on}}'}{(1 + k_{\text{on}}'/k_{\text{off}})^Q}.$$  

As in Eq. 2, we see that the exponential term in Eq. 4 also brings about the multiplicity of the Arrhenius factor for the single-site binding constant.

This newly introduced detail of the model reduces the calculated velocity significantly for temperatures above 25°C (solid line in Fig. 3 A). With much smaller facilitating force, the energy barrier associated with the unbinding of each site is larger, at 17 $k_B T$ (Table 1). Consequently, the weakly facilitated rate rises sharply, starting at ~25°C (Fig. 3 C). Nevertheless, it is smaller than the peel-off rate because of less facilitation.

**The load-velocity curve explains the dynamical trajectory**

In this section we show an interesting hysteresis behavior in the load-velocity curve at low velocities. This leads to an explanation for the dynamical trajectory observed in optical trap experiments.

The calculated load-velocity curve of the model fits with the experiment data (Fig. 4 A). Our calculation also extends the left end. The whole foot peel-off rate, $R_p$, has a much larger Arrhenius factor than the off-rate of a single site does because of the multiplying effect shown in Eq. 2. Also, the Arrhenius factor of $R_p$ decreases as temperature increases. (C) The peel-off rate $R_p$ and weakly facilitated release rate $R_{\text{wf}}$. The weakly facilitated rate becomes significant at ~25°C, resulting in the attenuation of velocity at high temperatures.
TABLE 1 List of parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Physical meaning</th>
<th>Source/reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>100</td>
<td>Number of legs.</td>
<td>Out of a total of 400 legs, one-quarter face the substrate.</td>
</tr>
<tr>
<td>Q</td>
<td>10</td>
<td>Number of binding sites on each foot.</td>
<td>Structural information: number of charges on the foot.</td>
</tr>
<tr>
<td>$f_m$</td>
<td>0.39 pN</td>
<td>Motor force.</td>
<td>Fitting Eqs. 1 and 2 with T-V data.</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>28 nm</td>
<td>Power stroke length.</td>
<td>Structure of leg protein: 90° conformational change between two arms of 20 nm.</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>80 P N/\mu m</td>
<td>Elastic constant of intermediate segment.</td>
<td>Physiological range.</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>$2.9 \times 10^9$ s$^{-1}$</td>
<td>Binding rate of single site.</td>
<td>Fitting Eqs. 1 and 2 with T-V data. assuming that the on-rates are not affected by external force.</td>
</tr>
<tr>
<td>$k_{off}$</td>
<td>$4.2 \times 10^1$ s$^{-1}$($10.6 k_B T$)*</td>
<td>Peel-off rate of single site (and its Arrhenius factor).</td>
<td>Fitting Eqs. 1 and 2 with T-V data.</td>
</tr>
<tr>
<td>$k_{off}'$</td>
<td>$1.9 \times 10^3$ s$^{-1}$($16.8 k_B T$)*</td>
<td>Weakly facilitated release rate of single site (and its Arrhenius factor).</td>
<td>Fitting Eqs. 1 and 2 with T-V data.</td>
</tr>
</tbody>
</table>

*Rates are listed as their values at the reference temperature 22.5°C.

The dynamical trajectory observed in the experiment, in which the Mycoplasma is attached to an optically trapped bead. This experiment captures the slowing down of the motion to near-stall (i.e., zero velocity), however, the spontaneous foot release has to be taken into account. It circumvents the peel-off of the foot and the negative impulse associated with it. This enhanced model is formulated via the transport equations in Section III of the Supporting Material. The solution gives the load-velocity curve in Fig. 4A.

The hysterisis in the load-velocity curve explains the dynamical trajectory observed in the experiment, in which the Mycoplasma is attached to an optically trapped bead. This experiment captures the slowing down of the motion to near-stall (Fig. 5 in Miyata et al. (23); also shown in the inset of Fig. 4B). At first, the cell drags the trapped bead away from the center of the laser beam, thus increasing the load force experienced (the laser trap is well approximated by a quadratic potential, i.e., a linear spring). The cell slows until it reaches the position in the trap that generates a load force ~20 pN. At this force, the cell begins to slide backward, and eventually breaks off from the substrate. Then the cell is quickly drawn back to the center of the trap where it reattaches, and the cycle repeats.

The corresponding trajectory is mapped out on the load-velocity curve, shown in Fig. 4B. The cell first traces down the upper branch of the load-velocity curve until it reaches the nose. It cannot follow the unstable middle branch—otherwise it would have a positive velocity yet move in the backward direction with decreasing load force. Thus, it must jump to the lower branch of the curve. On this branch, the cell begins to slide back (negative velocity). However, this does not persist long before the cell detaches from the substrate. Now the cell is quickly drawn back to the center of the trap with a much larger velocity determined by its hydrodynamic drag.

The stall force changes with temperature much less than the unloaded velocity. At very low velocity, the feet hardly complete the power stroke before the weakly facilitated release. For the same reason, the peel-off is interrupted by the spontaneous foot release. Therefore, the average force contributed by a foot in an average cycle is approximately the motor force multiplied by the fraction of cycle period spent in the power stroke. The motor force is insensitive to temperature, as discussed previously. The fraction of power stroke time apparently does not change too much with temperature (solid line in Fig. 5B), either, nor does the stall force.

However, the stall force predicted by the model is somewhat smaller than the stall force measured by the optical trap experiment. This may be due to the approximation that, during the peel-off, the unbinding rates of all the binding sites are assumed identical. In reality, the unbinding rates probably increase with the order of sites because the tension of the leg increases with time. However, without further information about the elasticity and geometry of the leg, it is fruitless to pursue the model beyond its current stage.

DISCUSSION

We have constructed a minimal mechanochemical model of the Mycoplasma motility apparatus based on current knowledge. The model is able to explain the interesting biophysical properties of the motility, especially the steep, sub-Arrhenius dependence of velocity on temperature. The model assumes the simplest coupling among the motor, intermediate segment, and foot, and no coupling between legs. Each leg simply rows forward and backward which, if completely symmetric, would not produce any net forward motion. Net forward motion is guaranteed by the asymmetric geometry of the leg, which causes the foot to release from the substrate more easily when peeled from the back. The high temperature sensitivity of the peel-off rate results from the multiplicity of the single-site Arrhenius factor. The factor decreases as temperature rises, contributing to the sub-Arrhenius behavior of the temperature-

Biophysical Journal 97(11) 2930–2938
velocity curve. Furthermore, the weakly facilitated foot release during the power stroke curtails the positive impulses and reduces the velocity at high temperatures. Finally, the dynamical process measured in laser trap experiments is explained qualitatively by the resultant load-velocity curve.

Certain biological parameters are estimated through the model. The binding and release rates of single binding site directly result from the fitting to the experimental data. They are all \( \sim 10^3 \) Hertz (Table 1). The peel-off rate is 2–3 times as large as the weakly facilitated release rate. The average cycle period of the leg in the unloaded cell is approximately \( 10-10^2 \) ms, which shortens with increasing temperature (Fig. 5 A). The power stroke and the subsequent leg restretching each takes \( \sim 40\% \) of the period in average (Fig. 5 B). Peeling off the foot takes \( \sim 15\% \) of the period. For the rest of the cycle period, the foot is unbound. Notice that the fraction of unbound time increases significantly with temperature, which helps explain that the cell easily detaches from the substrate at high temperatures. The cycle period also increases with increasing load force or decreasing velocity. When the cell approaches stall, the cycle is limited by the spontaneous...
The Stokes efficiency, estimated by $F_L V\tau/\Delta G_{\text{ATP}}$, is $\sim 10\%$ for the optimal load; here, $F_L$ is the load force and $\tau$ the cycle period. The above estimations fall in the proper biological range. Nevertheless, they are very rough, limited by the coarse-graining of the model.

The model also provides several predictions for experimental comparison. For example, the cell velocity peaks at a certain sialic acid density on the substrate (Fig. 6 A). This was shown by experiments changing the concentration of sialic acid used for coating the substrate (compare to Fig. 6 in (30)). Only qualitative comparison can be drawn at this moment, because we lack detailed information about the mechanism of foot binding as well as the relationship between sialic acid concentration in the coating medium and the sialic acid density finally presented on the surface of the substrate. We can also predict the effect of medium viscosity on the load-velocity curve (Fig. 6 B). Increasing the viscosity reduces the velocities for any given load force. In addition, the load-velocity appears more concave at higher medium viscosity. This change occurs because the resetting process is slowed at high viscosity and the hydrodynamic drag forces become more significant, compared with the other forces involved in the motility. Consequently, the cycle period is lengthened and the net force impulse provided per cycle is affected as well (compare to Eq. S28, Eq. S30, Eq. S33, and Eq. S34 in the Supporting Material).

Single molecule experiments on single legs are probably the best way to test the foot-substrate interaction mechanism proposed here. According to our model, the leg should break off from the substrate much easier under forward pulling force than it does under backward pulling force. Further structural information on the binding sites would also be useful in narrowing down the range of model parameters and estimating the energy barrier involved in the unbinding process.

The model for *Mycoplasma* motility can be generalized to other motility systems. Many walking molecular motors such as kinesin and myosin, show steep, sub-Arrhenius temperature-velocity-curves (25,26,32–34). Even in rotary motors, like the *Escherichia coli* flagellar motor (35,36), the way that the stators push on the rotor is analogous to the *Mycoplasma* legs walking on the substrate. Unlike the intuitive considerations of the catalytic biochemistry, we proposed the motor-substrate interaction as the major factor to explain the temperature sensitivity. This theory can be more easily tested with experiments on molecular motors because there are more techniques to manipulate them.

Limited by the information on *Mycoplasma* motility, our model is quite coarse-grained with many approximations. The peel-off mechanism itself was derived with the approximation of identical off-rates for all binding sites. A refined model with more realistic description of the rates can probably fit the stall force better. Moreover, the model is built on the horizontal spatial coordinate only. The vertical components of the forces, however, probably play a critical role in the peel-off of the foot, and even the break-off of the whole cell. Adding such details requires a more refined model. However, without knowing the detailed molecular mechanism of the foot-substrate interaction, such elaboration is merely guesswork.

**APPENDIX: HIDDEN COORDINATES FOR THE MOTOR**

The molecular motor is a large protein, and so a complete atomic description of its motion requires a very high-dimensional configuration space: at least 3N-dimensional, where N is the number of atoms. Generally, the large-scale motions of proteins are dominated by a small number of modes. For example, we have simplified the motion of the motor to the opening and
closely projecting the proximal part of the leg protein. By doing so, we are essentially projecting the high-dimensional periodic motion onto a single coordinate and treating it as a one-dimensional periodic oscillation. Although the resolved motor cycle appears to be moving forward and then backward along exactly the same trajectory, the hidden atomic degrees of freedom do not exactly retrace the same route. In particular, the second law of thermodynamics requires a loop in the force-displacement phase trajectory to account for the free energy consumption in the biochemical process. Such a loop is impossible when we simplify the motion to one dimension. We account for the free energy consumption in the biochemical process. Such dynamics requires a loop in the force-displacement phase trajectory to specifically projecting the high-dimensional periodic motion onto one single coordinate, say \((z, \theta)\), to form a cyclic loop in the \((z, \theta)\) plane. Therefore, the open conformation of the motor before the power stroke and the open conformation after the ADP release, although not distinguished in the model, are in general not equivalent. ATP can bind to the former conformation, but not the latter one. Instead of increasing the dimensionality of the model, we simply declare that ATP loading only happens after the leg fully resets to the front position.

**SUPPORTING MATERIAL**

Three supplementary sections with one table, one figure, and 34 equations are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)01466-0](http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)01466-0).

**REFERENCES**

Supporting Material

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

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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:

\[
Q \xrightarrow{k_{\text{on}}/k_{\text{off}}} Q \xrightarrow{k_{\text{on}}/k_{\text{off}}} \cdots \xrightarrow{k_{\text{on}}/k_{\text{off}}} 0 \text{ (all-off)}
\]

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 \(j\).

![Diagram of Markov chain](image)

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

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

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

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

(S1)

Rearranging the above equation and canceling the common factor \(dT\) yields

\[
\sum_{j \neq i} T_j r_{ij} - T_i \sum_{j \neq i} r_{ij} = -1
\]  

(S2)

Eq.(S2) can be written in vector form as

\[
P^T \mathbf{T} = -1
\]  

(S3)

where \(\mathbf{T} = \{T_i\}_{i=0}^Q\) is the vector of MFPTs. Note that \(T_0 = 0\), 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, \(P\).
\[ \begin{bmatrix} -k_{on} & k_{off} \\ k_{on} & -k_{on} - k_{off} & k_{off} \\ -k_{on} - k_{off} & k_{off} & \ddots \\ \vdots & \ddots & \ddots & \ddots \\ k_{on} & \cdots & k_{off} \\ -k_{on} - k_{off} & k_{off} & \vdots \\ k_{on} & -k_{off} \end{bmatrix} \]

\( P \)

\( P \) 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:

\[
-k_{off} T_{i-1} + k_{on} T_{i+1} - (k_{on} + k_{off}) T_i = -1, \quad i = 1, \ldots, Q-1
\]

(S5)

\[
k_{off} T_{Q-1} - k_{off} T_Q = -1
\]

(S6)

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

\[
k_{on} \Delta T_i - k_{off} \Delta T_{i-1} = -1, \quad i = 1, \ldots, Q-1
\]

(S7)

\[-k_{off} \Delta T_{Q-1} = -1 \Rightarrow \Delta T_{Q-1} = 1/k_{off}
\]

(S8)

Let \( \Delta T'_i := \Delta T_i - \frac{1}{k_{off} - k_{on}} \), then Eq. S7 and Eq. S8 are equivalent to

\[
\Delta T'_{i-1} = K \Delta T'_i
\]

(S9)

\[
\Delta T'_{Q-1} = -\frac{K}{k_{off} - k_{on}}
\]

(S10)

where \( K = k_{on}/k_{off} \).
Eq.S9 and Eq.S10 give

\[
\Delta T'_i = K^{Q-i} \Delta T'_{Q-1} = -\frac{K^{Q-i}}{k_{off} - k_{on}}
\]  

(S11)

Thus,

\[
T_Q = T_0 + \sum_{i=0}^{Q-1} \Delta T_i
\]

\[
= \sum_{i=0}^{Q-1} \left( \Delta T'_i + \frac{1}{k_{off} - k_{on}} \right)
\]

\[
= \sum_{i=0}^{Q-1} \left( 1 - K^{Q-i} \right)
\]

\[
= \frac{Q}{k_{off} - k_{on}} - \frac{1}{k_{off}} \left( Q + 1 - \frac{1 - K^{Q+1}}{1 - K} \right)
\]

\[
= \frac{1}{k_{off}} \frac{Q - (Q+1)K + K^{Q+1}}{(1 - K)^2}
\]

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

\[
R = k_{off} \frac{(1 - K)^2}{Q - (Q+1)K + K^{Q+1}}
\]  

(S12)

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

\[
Q \xleftarrow{k_{off}} Qk \xrightarrow{k_{on}} Q-1 \xleftarrow{\frac{k_{off}}{2k_{on}}} (Q-1)k \xrightarrow{\frac{2k_{off}}{(Q-1)k_{on}}} \cdots \xleftarrow{\frac{k_{off}}{Qk_{on}}} 1 \xrightarrow{k_{off}} 0 \quad \text{(all-off)}
\]

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

\[
r_{i-1 \rightarrow i} = (Q - i + 1) \cdot k_{on}
\]

\[
r_{i \rightarrow i-1} = i \cdot k_{off}
\]  

(S13)

and the transition matrix is
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, \( \tau_u = \frac{1}{k_{on}} \), \( \tau_b = \frac{1}{k_{off}} \), respectively. The probability of finding a site in the unbound state is

\[
p_u = \frac{\tau_u}{\tau_b + \tau_u}
\]

(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

\[
\frac{T_{off}}{T_{on} + T_{off}} = p_u^Q = \left( \frac{\tau_u}{\tau_b + \tau_u} \right)^Q
\]

(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 \( T_{off} = \frac{1}{Qk_{on}} = \frac{\tau_u}{Q} \) into Eq.S16 yields

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

(S17)

Then the reciprocal of \( T_{on} \) is the foot unbinding rate, same as Eq.4 in the main text.
\[ R = \frac{Q/\tau_u}{(1 + \tau_b/\tau_u)^Q - 1} = \frac{Q k_{on}}{(1 + k_{on}/k_{off})^Q - 1} \] (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 4A 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 \( f_m/\zeta_f \), where \( f_m \) is the motor force, and \( \zeta_f \) the hydrodynamic drag coefficient of the foot. The ensemble density of these feet is denoted \( \rho_1(x) \), in \( 0 = x \leq \lambda \). 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:

\[ V \frac{d}{dx} \rho_0 = -R(x) \rho_0 \] (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 \( R(x) \):

\[ R(x) = \begin{cases} 
R_{wf}(x), & 0 \leq x < \lambda \\
R_s(x), & \lambda \leq x < \lambda + L \\
R_p(x), & x \geq \lambda + L 
\end{cases} \] (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 $0 \leq x < \lambda$ is based on translational velocity $\dot{x} = f_m / \zeta_f$, and the source due to foot release in $0 < x < \lambda$.

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

(S21)

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

$$
\rho_1(0) = 0
$$

(S22)

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

$$
-\frac{f_f}{\zeta_f} \frac{d\rho_2}{dx} = \begin{cases} 0, & 0 \leq x < \lambda \\ R(x)\rho_0, & x \geq \lambda \end{cases}
$$

(S23)

Finally, we have two flux balance boundary conditions:

$$
V\rho_1(0) = \frac{f_f}{\zeta_f} \rho_2(0)
$$

(S24)

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

(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:

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

(S26)

Assume all foot release rates are invariant with position. Then Eqs.S19-S26 can be solved analytically with solutions:
where $T$ is the average duration of the whole cycle.

$$
T = -\frac{\exp\left(-\frac{R_s x}{V}\right)}{R_{nf}} + \frac{1 - \exp\left(-\frac{R_s L}{V}\right)}{R_s} + \frac{\exp\left(-\frac{R_s L}{V}\right)}{R_p} + \frac{\zeta f_m}{f_r} \left(1 - \frac{1 - \exp\left(-\frac{R_s \lambda}{V}\right)}{R_{nf}} \right) + \frac{\zeta f_m}{f_r} \left(1 - \frac{\exp\left(-\frac{R_s L}{V}\right)}{R_s} + V \frac{\exp\left(-\frac{R_s L}{V}\right)}{R_p}\right)
$$

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
\[
\left( F_L + \zeta V \right) / N = f_m \int_0^\lambda \rho_0(x) dx - f_i \int_{\lambda+L}^{\lambda+L} \rho_0(x) dx - \int_{\lambda+L}^{\infty} \kappa (x - \lambda - L) \rho_0(x) dx \\
+ f_m \int_0^\lambda \rho_1(x) dx - f_i \int_0^{\lambda} \rho_2(x) dx
\]  
(S29)

Plugging in Eq.S27 gives

\[
F_L = -\zeta V + \frac{N}{T} \left\{ \frac{f_m - \zeta V}{R_{wf}} \left( 1 - \exp \left( -\frac{R_{wf} \lambda}{V} \right) \right) - \frac{\left( \kappa / R_p - \zeta f \right) V}{R_p} \exp \left( -\frac{R_L}{V} \right) + \frac{f_i - \zeta V}{R_s} \left( 1 - \exp \left( -\frac{R_L}{V} \right) \right) \right\}
\]  
(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 \( \zeta_f, \zeta_b, R_s, f_i \to 0 \). 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 \( \rho_1 \) represents the density of the foot that has been spontaneously released during re-stretching. \( \rho_2 \) corresponds to the foot that has been snatched off the substrate during the powerstroke. The governing transport ODEs are given in Eqs.S31.
\[ V \frac{d \rho_0}{dx} = -R(x) \rho_0, \quad (a) \]
\[ -\frac{f_r}{\zeta_f} \frac{d \rho_1}{dx} = R(x) \rho_0, \quad 0 < x \leq \lambda \quad (b) \]
\[ \frac{f_m \frac{d \rho_2}{dx}}{\zeta_f} = \begin{cases} R(x) \rho_0, & x \leq 0 \\ 0, & 0 < x \leq \lambda \end{cases} \quad (c) \]
\[ -V \rho_0(\lambda) = \frac{f_m}{\zeta_f} \rho_2(\lambda), \quad (d) \]
\[ -V \left( \rho_0(0^-) - \rho_0(0^+) \right) = \frac{f_r}{\zeta_f} \rho_1(0), \quad (e) \]
\[ \rho_1(\lambda) = 0, \quad (f) \]
\[ \int_{-\infty}^{\lambda} \rho_0 dx + \int_{0}^{\lambda} \rho_1 dx + \int_{-\infty}^{0} \rho_2 dx = 1, \quad (g) \]

with the piecewise foot release rate
\[ R(x) = \begin{cases} R_{sf}(x), & x \leq 0 \\ R_r(x), & 0 < x \leq \lambda \end{cases} \quad (S32) \]

The meanings of each equation above are similar to those for positive velocities. The resulting load force as a function of velocity is
\[ F_L = -\zeta_V V + \frac{N}{T} \left[ f_m - \frac{\zeta_f V}{R_{sf}} - \frac{\kappa V}{R_s^2} - \frac{f_r + \zeta_f V}{R_r} \right] \left( 1 - \exp \left( \frac{R_s \lambda}{V} \right) \right) \quad (S33) \]

where the average cycle duration is
\[ T = \frac{1}{R_{sf}} + \frac{1 - \exp \left( \frac{R_r \lambda}{V} \right)}{R_s} + \frac{\zeta_f}{f_m} \left( \lambda - \frac{V}{R_{sf}} \right) + \frac{\zeta_f}{f_r} \left( \lambda + \frac{1 - \exp \left( \frac{R_s \lambda}{V} \right)}{R_r} \right) \quad (S34) \]

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. \( \rho_0 \) dominates over \( \rho_1 \) and \( \rho_2 \) 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, \( \rho_1 \) at \( V > 0 \) is extremely small because the foot is released with the weakly facilitated rate \( R_{sf} \), and then driven fast by the relatively large force \( f_m \). By contrast, \( \rho_2 \) 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Physical meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>25 nm</td>
<td>distance between the end of powerstroke and backward re-stressed position</td>
</tr>
<tr>
<td>$f_r$</td>
<td>0.005 pN</td>
<td>weak resetting force</td>
</tr>
<tr>
<td>$\zeta_f$</td>
<td>200 pN·s/m</td>
<td>hydrodynamic drag coefficient of the foot</td>
</tr>
<tr>
<td>$\zeta_b$</td>
<td>$2 \times 10^4$ pN·s/m</td>
<td>hydrodynamic drag coefficient of the cell body</td>
</tr>
<tr>
<td>$k_{\text{off}}$</td>
<td>$1.7 \times 10^3$ s$^{-1}$ (16.8 $k_B T$)</td>
<td>spontaneous release rate of single site (and its Arrhenius factor)</td>
</tr>
</tbody>
</table>
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 ($\rho_0$), released during the powerstroke with weak facilitation ($\rho_1$), and spontaneously released or peeled off after the powerstroke ($\rho_2$). 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 ($\rho_0$), spontaneously released during the post-power-stroke relaxation ($\rho_1$), and snatched off during the powerstroke ($\rho_2$). The diagrams below the cartoons show examples of typical density distribution of the feet at different states at 22.5°C.