The lawnmower: an artificial protein-based burnt-bridge molecular motor

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Abstract

Molecular motors are protein-based machines essential for directional transport of cellular components. Inspired by biology, in this work we synthesize and characterize a protein-based microscale motor we dub the lawnmower. It is comprised of a spherical hub decorated with trypsin enzymes; its motion is directed by cleavage of a peptide lawn, which promotes motion towards fresh substrate. We find the lawnmower is capable of superdiffusive motion, and can attain average speeds of up to 80 nm/s. By contrast, the lawnmower exhibits exhibit purely diffusive motion on lawns lacking the peptide substrate. We believe the lawnmower is the first example of an autonomous protein-based synthetic motor purpose-built using nonmotor protein components.

Keywords: Molecular motor, Burnt-bridges ratches, Mean squared displacement, Anomalous diffusion, Superdiffusion
1 Introduction

Molecular motors are essential for powering directional motion at the cellular level, including transport and sorting of cargo, cell locomotion and division, and remodelling of the extracellular environment [1, 2]. Such molecular motors are made out of proteins whose directed motion is coupled to the consumption of chemical free energy. Inspired by such biological machines, significant strides have been made to design and implement synthetic devices capable of directed motion on the nanoscale [3–10] and the microscale [11–13]. While these have been impressive achievements, thus far, directed motility of a synthetic protein-based motor has not been demonstrated.

Novel motor proteins have been crafted by swapping or re-engineering domains of natural protein motors [14–18]; however, these have used motor components in their construction. Distinct ideas have been proposed for using non-motor proteins as building blocks to engineer novel motors [18–21], but to our knowledge motility of these non-motor-containing concepts has not been achieved.

In this work, we demonstrate biased motion of a protein-based motor dubbed the lawnmower. Distinct from previous synthetic designs, the lawnmower uses neither natural motor-protein components nor DNA, in the motor nor track. The lawnmower is designed to operate as a burnt-bridges ratchet, whereby it exploits chemical free energy consumption and polyvalency to drive its directed motion on a two-dimensional surface.

2 Results

2.1 Lawnmower concept and design

Nature has exploited many mechanisms to achieve directed motion at the nanoscale. Our design utilizes the burnt-bridge Brownian ratchet (BBR) mechanism. In the Brownian ratchet mechanism, motion is driven by thermal fluctuations with direction biased by chemical reactions that induce local spatial asymmetry [22, 23]. A burnt-bridges design exploits cleavage or removal of surface-bound free-energy-rich substrate sites to induce this spatial asymmetry; a pure burnt-bridges ratchet has no ability to bind to its burnt (cleaved) track [24, 25].

The BBR mechanism appears to power directed motility in a range of natural systems, including eukaryotic cell migration [26], plasmid partitioning in bacteria [27, 28], bacterial engulfment [29], pericellular viral motion [30, 31], and enzymatic processing of the abundant structural biopolymers collagen [32, 33], cellulose [34], and chitin [35, 36].

Synthetic BBRs have been created that use DNA to achieve motion at the nanoscale [6–8, 37, 38] and microscale [12, 13, 39]. However, to our knowledge, motility of a synthetic protein-based BBR has not been achieved. Inspired by the BBR mechanism of collagenase [32], we designed a protease-based BBR we call the lawnmower [20].
As shown schematically in Figure 1, the lawnmower (LM) consists of trypsin proteases coupled through a central microspherical hub; these protease “blades” bind to and cleave peptide substrates presented via an F127 polymer brush adhered to a glass coverslip [40]. Once the LM lands on the surface, diffusion of the central hub allows unbound protease blades to engage nearby peptides in any direction. However, the initial cleavage and diffusion direction breaks symmetry. The lack of peptides behind the LM creates a free energy gradient and biases diffusion of the LM towards uncleaved peptide “grass” (Figs. 1, S1). We expect to see LM dynamics on a peptide lawn exhibiting this biased motility (similar to a self-avoiding walk), whereas on a lawn lacking peptides, the LM is expected to exhibit diffusive dynamics [41].

Fig. 1 The lawnmower molecular motor concept based on a burnt-bridge ratchet (BBR) design. The motor consists of trypsin proteases (yellow) linked to a central microsphere hub (green). Cleavage of peptide substrates (“grass”; red triangles) results in products (pale blue), and a free energy preference for trypsin to bind intact peptides. This results in biased, directional motion of the lawnmower on the surface.

2.2 Lawnmower trajectories

We observe striking differences between trajectories of LMs on a peptide lawn (peptide-F127) and on a peptide-free, bare lawn (F127), as shown in Fig. 2a,b. Over similar timescales LMs on peptide lawns travel much farther than on bare lawns. This agrees with the designed BBR mechanism, whereby a spatial asymmetry arising from peptide cleavage allows the LM to achieve more directional motion. Directional bursts can be seen on the peptide lawn, which are not observed on the bare lawn. These provide additional evidence of distinct peptide-dependent dynamics.

A further distinction between the peptide and bare lawns was the observation of non-motile LMs on peptide lawns. While the majority of LMs were motile on peptide lawns (55%; \( n = 59 \)), a significant fraction appeared to remain stuck throughout the 12.5-hour experiment (\( n = 49 \)). We classified a LM as motile if its mean-squared displacement exceeded a threshold value
(Fig. S2a), and these are the LMs whose dynamics we analyse throughout this work.

Non-motile trajectories may be those that have adhered to the glass cover slip, having found a barren patch where no lawn is laminated or where the lawn is less dense. The LM may also non-specifically bind the peptide F127 that leads to the motor sticking. The hypothesis that non-motile LMs occur because of a peptide interaction is supported by there being essentially no non-motile LMs in the bare F127 control experiment (<1% across all replicates). Trajectories of non-motile LMs, when plotted from a common origin, overlap, and were therefore used to determine the average drift trajectory (Fig. S2b), which was then used to correct for drift in the motile trajectories.

### 2.3 Lawnmower mean-squared displacements

To more quantitatively assess the dynamics of the lawnmowers on the two types of surfaces, we determined the time dependence of the LM mean-squared displacement (MSD). The MSD is proportional to the time lag to the power $\alpha$, where $\alpha$ is the anomalous diffusion exponent:

$$\text{MSD} \propto \tau^\alpha. \quad (1)$$

The anomalous diffusion exponent characterizes the type of diffusion [42–44]. Subdiffusion, conventional diffusion, and superdiffusion correspond to $0 \leq \alpha < 1$, $\alpha = 1$, and $1 < \alpha < 2$, respectively, while for a purely ballistic system proceeding at constant velocity, $\alpha = 2$. Thus, for LMs on bare F127 surfaces, we expect conventional diffusion with $\alpha = 1$, while for peptide-fueled BBR dynamics, we anticipate $\alpha > 1$.

For each trajectory, we determined its trajectory-averaged MSD, $\text{MSD}_{TA}$ (Eq. 4), as a function of time lag $\tau$. It is immediately apparent that LMs on peptide lawns exhibit far more heterogeneous dynamics than those on bare lawns (Fig. 2c,d). Additionally, the slope of most MSDs on peptide lawns is not constant, showing subdiffusive character at very short times and increasing for longer time lags. In contrast, on the bare lawns, LM dynamics are significantly more homogeneous and are conventionally diffusive, with $\alpha \approx 1$.

Although there is significant heterogeneity of LM dynamics on peptide lawns, we begin by presenting an average $\alpha$ for each LM trajectory, via a linear fit of its $\log(\text{MSD}_{TA})$ vs $\log(\tau)$ over all time lags from $\tau = 10$ s to $\tau_{\text{max}} = 0.1T_{\text{msr}}$, representing 10% of the total measurement time of 12.5 hours for the peptide lawn and 6.2 hours on the bare lawn. The resulting histograms of $\alpha$ values are shown in Fig. 2e for all motile LMs on peptide lawns and in Fig. 2f for LMs on bare lawns. Using this approach to compute an effective $\alpha$ for each LM, we find motile LMs on peptide lawns to display a range of anomalous diffusive dynamics ranging from subdiffusive ($\alpha = 0.5$) to superdiffusive ($\alpha = 1.4$). By contrast, LMs on bare lawns exhibit $\alpha$ values clustered more closely around $\alpha = 1$, exemplifying more conventionally diffusive dynamics. In the Supporting
Fig. 2 Trajectory-based analysis of lawnmowers on peptide and bare lawns. (a) Sample trajectories of motile LMs on a peptide lawn. $n = 8$ representative trajectories in (a) and (b) are plotted on the same spatial scale (inset in (b) is enlarged), over the same duration (6.2 hours), and are shifted to a common origin. (c) MSD$_{TA}$ (Eq. 4) as a function of time lag for each of the trajectories shown in panel (a), though computed over the full 12.5-hour experimental duration. (d) MSD$_{TA}$ as a function of time lag for each of the trajectories shown in panel (b). Colours in (c,d) correspond to the trajectories in (a,b). (e) Histogram of $\alpha$ computed from a linear fit to the entire MSD$_{TA}$ curve from each motile LM moving on a peptide lawn ($n = 59$). (f) Histogram of the anomalous diffusion exponent ($\alpha$) computed from a linear fit to the entire MSD$_{TA}$ curve from each LM on a bare lawn ($n = 55$). Dashed lines at $\alpha = 1$ indicate what is expected for conventional diffusion.

Information, we discuss potential reasons for a slightly subdiffusive average value of $\langle \alpha \rangle = 0.95$ found for LMs on the bare lawn.

To determine LM ensemble dynamics on peptide and bare lawns, we calculated the ensemble average of the trajectory-based MSDs, MSD$_{ETA}$ (Eq. 5), for LMs on each surface. These results show a striking distinction in dynamics on
the two lawns (Fig. 3). The ensemble of LMs on bare lawns exhibits an approximately constant slope of $\alpha \approx 1.0$, confirming diffusive dynamics at all time lags. (Slight curvatures in slope around $\sim 200$ seconds may indicate sample drift; the lack of non-motile LMs on bare lawns prevented drift correction as done for LMs on peptide lawns. See Supporting Information for further discussion.) By contrast, LMs on peptide lawns exhibit subdiffusive dynamics at short time lags, progressing to superdiffusive dynamics from $\tau \sim 50$ s to $\sim 500$ s, followed by dynamics that approach conventional diffusion at much longer time lags. Such dynamics are consistent with a system that dwells at its current location for a time while cleaving substrate, then exploits this local free energy gradient at the substrate-product boundary to drive directed motion [41]. At times when displacements have become longer than trajectory persistence lengths, the dynamics transition to a weakly self-avoiding walk [41]. The MSD analysis demonstrates that LMs exhibit distinct dynamics on peptide and bare lawns, which corroborate their designed function.

![MSD plot](image)

**Fig. 3** Ensemble-averaged MSD analysis. MSD$_{\text{ETA}}$ (Eq. 5) is shown as a function of time lag for the LMs on peptide lawns shown in Fig. 2c (blue circles; $n = 8$) and for the LMs on bare lawns shown in Fig. 2d (red squares; $n = 8$). Solid lines indicate the region over which the anomalous diffusion exponent, $\alpha$, was computed.

### 2.4 Lawnmower displacement distributions

In agreement with the significantly larger MSD for LMs on peptide lawns, we find that LMs can exhibit a much greater range of displacements on peptide lawns than on bare lawns. Fig. 4a and Fig. 4c display displacements $\Delta r$ (over 10-s intervals) as a function of time for an example LM on a peptide lawn and on a bare lawn, respectively. ($\Delta r$ traces for all other trajectories shown...
in Fig. 2a and Fig. 2b are provided in Figures S3 and S4, respectively.) It is immediately clear that the LM on the peptide lawn exhibits bursts of longer-range displacements, while the LM on the bare lawn exhibits only shorter-range motion.

![Fig. 4 Displacement distributions of lawnmowers. (a) $\Delta r$ (over $\Delta t = 10$ s) versus time for a LM on a peptide lawn (green trajectory from Fig. 2a). The LM transitions stochastically between a long-range travel mode and strongly localized dynamics. (b) Histogram of displacements shown in panel (a). Inset: $P(\Delta r)$ on a semi-log scale. (c) $\Delta r$ versus time for a LM on a bare lawn (green trajectory from Fig. 2b). (d) Histogram of displacements shown in panel (c). Red line is a fit with the Rayleigh distribution (Eq. 2).](image)

The displacement distributions of LMs on peptide and bare lawns are qualitatively distinct. On a peptide lawn, the displacement distributions of motile LMs are right-skewed with a long tail to large displacements (e.g. Fig. 4b). This is consistent with the picture of localized dynamics of the LM during cleavage interspersed with gradient-driven runs. By contrast, the displacement distributions for LMs on bare lawns (e.g. Fig. 4d) are well fit by the Rayleigh distribution for two-dimensional diffusion (Eq. 2).

For each LM on a bare lawn, we fit its $P(\Delta r)$ as a function of time interval $\Delta t$ to obtain its diffusion coefficient (Eq. 2). The resulting diffusion coefficients
The lawnmower: an artificial protein-based burnt-bridge molecular motor

8 of LMs on bare lawns are tightly clustered, with \( \langle D_{\text{bare}} \rangle = 0.056 \pm 0.004 \, \mu m^2 \cdot s^{-1} \) \((n = 55; \text{error represents standard deviation})\); see Fig. S5. This value is somewhat less than the diffusion coefficient predicted for these spheres in free solution, as expected from their proximity to the surface (Supporting Information).

2.5 Lawnmower speeds

The distributions of 10-second interval speeds (Eq. 3) for all motile LMs on peptide lawns and for all LMs on bare lawns are shown in Fig. 5a. In agreement with what was seen for the example LMs in Fig. 4, the ensemble of LMs on peptide lawns has a broader distribution of speeds with significantly more weight at high speeds than LMs on bare lawns. The mean interval speed of the motile ensemble on peptide lawns is \( \langle v \rangle = 61 \pm 20 \, \text{nm} \cdot \text{s}^{-1} \), while the mean interval speed of LMs on bare lawns is \( \langle v \rangle = 23 \pm 4 \, \text{nm} \cdot \text{s}^{-1} \) (errors are standard deviations). This indicates that lawnmowers travel substantially faster on peptide lawns.

\[ \langle v \rangle = 61 \pm 20 \, \text{nm} \cdot \text{s}^{-1}, \quad \langle v \rangle = 23 \pm 4 \, \text{nm} \cdot \text{s}^{-1} \]

By determining the mean speed of each individual LM, we find that motile LMs on peptide lawns have much greater trajectory-averaged speeds than on bare lawns: LMs are, on average, faster on peptide lawns than on bare lawns (Fig. 5b). LMs on bare lawns exhibit homogeneous dynamics, with trajectory-averaged speeds tightly clustered around 23 nm·s\(^{-1}\). This corresponds well with the homogeneous behaviour revealed by the MSD analysis (Fig. 2d). By
contrast, trajectory-averaged speeds of individual motile LMs on peptide lawns range from 20 to 80 nm·s\(^{-1}\), with 50% of LMs having trajectory-averaged speeds of \(\bar{v} = 75 \pm 5\) nm·s\(^{-1}\). The broader distribution of these mean speeds is not unexpected given the large heterogeneity in anomalous diffusion for LMs on peptide lawns (Fig. 2e).

3 Discussion

In this work, we have constructed micron-sized, protein-based devices we dubbed lawnmowers. Designed to act as burnt-bridge ratchets, the LMs display dynamics supporting this mechanism. We have found LM motility on peptide lawns to be characterized by time-dependent superdiffusion, in contrast with diffusive motion on bare lawns that lack the peptide substrates. LMs also exhibit significantly greater displacements on peptide lawns than on bare lawns, where displacement distributions are characterized by simple diffusion. While diffusion may be an effective means of exploring local space at short timescales, the coupling of chemical energy to directed motion allows molecular motors to travel much further at longer timescales than is possible under thermal diffusion. For the LM, we observe larger-range motion on peptide than bare lawns, even at our shortest observation times of 10 seconds. These dynamical characteristics all corroborate the design goal of a protein-based molecular motor.

We can compare the performance of the LM with other microscopic BBRs. The microsphere-based Par design of Vechhiarelli et al. represents an in vitro reconstitution of the BBR-type plasmid partitioning system in E.coli [39]. It uses proteins associated with a DNA-coated microsphere to stimulate ATP hydrolysis by lawn proteins bound to a surface of DNA; ATP hydrolysis results in lawn protein dissociation, thereby clearing a patch of substrate under the microsphere and creating a free energy gradient at its edges, directing preferential movement towards regions of an intact lawn. Par microspheres displayed directed trajectories, with an average speed of 100 nm/s. Somewhat lower average speeds of \(~20\) nm/s were achieved by a purely synthetic microscale BBR system, the “monowheel” [12]. This design used nucleic acid hybridization to bind a DNA-coated microsphere to an RNA lawn; selective hydrolysis of the DNA-bound RNA by the enzyme RNaseH (provided in solution) led to the removal of the RNA lawn under the microsphere, again producing a free energy gradient at its edges and resulting in preferential movement towards intact RNA substrate. The average speed \((\sim 60\) nm/s) of LMs on peptide lawns is comparable to these systems, Thus, protein-based LMs exhibit comparable speeds to these previous designs that exploited DNA in their construction.

All three types of microscale BBRs display heterogeneity in their dynamics. Monowheels exhibit clearly bimodal displacement distributions [12], described as entrapped and non-entrapped states. While trajectories in the Par system were generally persistent, saltatory dynamics were observed in this system as well [39, 45]. Here, the two states were described as quenched diffusion (when
many bonds were engaged) and driven motion (when a sufficiently small number of bonds were engaged to permit rapid motion) \cite{45}. This latter model demonstrated the importance of balancing chemical kinetic rates of multiple interacting tethers with the timescale of bead diffusion. Interestingly, saltatory dynamics have not been observed in monowheel-like designs implementing nanoscale hubs \cite{38, 46}, demonstrating the role that polyvalency can have in influencing motility \cite{47–49}.

LMs can occupy at least two distinct dynamical states, similar to previous synthetic microsphere-based systems. The displacement and speed distributions are not as clearly bimodal for LMs as for monowheels \cite{12}, nor as in previous models inspired by these systems \cite{41}.

Because of the inherent dynamical heterogeneity in these systems, various measures should be applied to assess biased motion. We used displacement and speed distributions and anomalous diffusion exponents to characterize LM dynamics, examining each time interval of each LM, the properties of each LM averaged over its trajectory, and the ensemble-averaged behavior of all LMs. Displacement distributions of LMs on bare lawns are well described by conventional two-dimensional diffusion (Figs. 4d), which is not the case for peptide lawns (Figs. 4b). The speed distributions (over 10-second intervals) display enhanced motility of LMs on peptide lawns (Fig. 5a). The trajectory-averaged interval speeds of individual LMs also clearly distinguish LMs on peptide and bare lawns: 95% of LMs on peptide lawns have greater average interval speeds than LMs on bare lawns, with a majority exhibiting at least three-fold greater interval speeds than possible with diffusion (Fig. 5b).

By contrast, trajectory-averaged anomalous diffusion does not provide this obvious distinction for LMs: only about a quarter of LMs are characterized by a trajectory-averaged superdiffusive $\alpha > 1$ (Fig. 2e,f). This is surprising because the ensemble-averaged MSD clearly distinguishes the anomalous diffusion of a sub-ensemble of LMs on a peptide lawn from diffusion (Fig. 3). At short time lags, LM motion is sub-diffusive, with dynamics constrained by the need to cleave peptides and establish a local asymmetry to drive motion. At intermediate time lags, LMs are superdiffusive, undergoing strongly biased motion that presumably arises from the ability to actively maintain and couple to an underlying peptide “front”. At the longest time lags, $\alpha$ decreases, consistent with a transition to self-avoiding dynamics that bias motion away from already-cleaved regions of the surface. Determining a single value of $\alpha$ across all timescales washes out these dynamics, resulting in the generally subdiffusive trajectory-averaged $\alpha$ values across the LM population (Fig. 2e). Such subdiffusive, trajectory-averaged $\alpha$ values have been discussed in other systems displaying saltatory dynamics \cite{50}.

The LM design lends itself to modular design alterations, which can be used to rationally explore the BBR mechanism of motility. Simulations have shown the effects of many design parameters on the dynamics of polyvalent BBRs: linker length and flexibility \cite{41, 45}, tunable for LMs in the molecular tether linking trypsin to the hub (e.g. different lengths of PEG or use of more
rigid linkers like DNA); kinetic rates, particularly the appropriate balance of binding, cleavage and diffusion [28, 45, 48], tunable for the LMs by the use of different proteases [20]; and polyvalency [47–49, 51, 52], tunable for LMs by controlling trypsin density on the sphere or by the use of different hubs such as quantum dots [20], streptavidin [7] or peptide assemblies [53]. It is not just the LM but also its track that combine to enable the BBR mechanism. Thus, changes in the peptide lawn can also alter dynamics, as shown through simulation: the stiffness of the peptide supports [41], here tunable from the F127 polymer brush to a DNA-based presentation [54] or more rigid covalent attachment to the glass [55]; the surface density of peptides on the lawn [41]; and the dimensionality of the lawn [48], which could be tuned by selective peptide patterning within confining 1D microchannels or, for nanoscale hubs, the use of peptide-labeled dsDNA tracks [54] or peptides positioned at prescribed locations on a DNA origami support [8]. The ability to control the positions of proteases and their substrates may also enhance homogeneity of LM dynamics.

In the future, insight on the mechanochemical coupling underlying the LM’s BBR mechanism could be obtained by the use of fluorescence to discriminate between substrates and products [12] and/or force-dependent linkages between the LM and track [13]. The peptides provided as substrates in these LM experiments bridge a fluorophore and quencher, whereby peptide cleavage releases the quencher to solution and the surface-bound products become fluorescent. In the current experiments, we used bright-field microscopy only, to track the dynamics of the LM, but the system is adaptable to fluorescence-based measurements as has been done to image the track generated by DNA-based polyvalent BBR designs [12, 38, 46].

Distinct from the related Par and synthetic monowheel microscale BBR systems [12, 39], the LM does not rely on the supply of reagents from solution (ATP and RNaseH, respectively) to sustain motion. Instead, the free energy of the LM system is supplied within its prepared peptide lawn. In this aspect, the LM bears more similarity to biological systems such as collagenases [32], cellulases [34] and chitinases [35], whereby the free energy to power directed motion comes from cleavage of the track itself. Like these biological BBRs, the LM operates autonomously to cleave and self-propel along its track.

4 Conclusion

In this work, we have synthesized and characterized the dynamics of the lawnmower, a microscale burnt-bridges ratchet. Our results demonstrate the peptide-dependent motility of this polyvalent protease-based design. LMs attain average speeds on their peptide tracks of \( \sim 60 \) nm/s However, their dynamics are heterogeneous. LMs are found to have saltatory motion, similar to DNA-based BBRs [45], and possess a non-diffusive speed distribution, similar to that of a distinct DNA-based synthetic system [12]. Dynamical heterogeneity of LMs is also apparent in their anomalous diffusion characteristics. By contrasting LM dynamics on a peptide and bare lawn, we see that
all of these dynamical features are peptide-dependent, thereby supporting the
designed BBR mechanism of motility.

5 Methods

5.1 Lawnmower preparation

The synthesis of microscale lawnmowers was adapted from a previous proto-
col for quantum-dot-based LMs [20]. Microscale LMs were built around M-270
amine Dynabead hubs (diameter 2.8 µm; ThermoFisher 14307D). Heterobi-
functional crosslinkers sulfo-SMCC (sulfosuccinimidyl-4-(N-maleimidomethyl)
cyclohexane-1-carboxylate, ThermoFisher A39268) were linked to the bead
and used to tether trypsins. Cysteines on trypsins were reduced with TCEP
(ThermoFisher 77712) prior to incubation with the Dynabead-Sulfo-SMCC
hub. Trypsin activity is retained throughout this treatment [20]. All reaction
conditions were as previously described [20], except that a magnet was used
to pellet beads during buffer exchanges and wash steps, and the incubation
times of Dynabeads with sulfo-SMCC, and of sulfo-SMCC-coated Dynabeads
with reduced trypsin were each extended to 4 hours. Further information
about lawnmowers, including estimates of sizes and densities of components,
are provided in the Supporting Information.

5.2 Lawn preparation

Substrate peptides were presented as a two-dimensional lawn on a glass cov-
erslip, on which they were supported by an F127 block-copolymer brush. The
preparation of the peptide and bare lawns is as previously described [40]. The
peptide lawn uses an NHS-functionalized F127 (Polymer Source P40768-
EOPOEO2NHS) to couple the central lysine of the peptide (sequence (N
to C) FITC-Ala-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys-DABCYL, custom order
from Biomatik) to the N-hydroxy-succinimidyl ester moiety on the F127. The
lawn is readily cleaved by trypsin presented free in solution [40]. The bare
lawn used standard F127 (Sigma P2443). The F127 brush of the bare lawn did
an outstanding job of blocking nonspecific adhesion of the LMs to the surface
(<1% immotile), consistent with its previously demonstrated blocking abilities
with microspheres of other surface chemistry [40].

5.3 Imaging and tracking lawnmower motion

Imaging was conducted using brightfield microscopy (Zeiss Axioskop with 10x
objective; FLIR Blackfly camera). Custom software was used to record image
frames at 10 second intervals throughout lawnmower experiments. Experi-
ments on peptide lawns were each run for a total of 4490 frames (12.5 hours),
while experiments on bare lawns were run for 2238 frames (6.2 hours). LM
density was sufficiently low to maintain non-overlapping trajectories through-
out an experiment. LM trajectories were determined from image stacks using
the Fiji plugin MTrack2 [56].
5.4 Analytical methods

Displacement distributions of LMs on bare lawns were fit with the Rayleigh distribution for 2D diffusion [57]

\[
P(\Delta r, \Delta t) = \frac{\Delta r}{2(D\Delta t + \sigma^2)} e^{-\frac{\Delta r^2}{4(D\Delta t + \sigma^2)}}.
\]  

(2)

Here, \(\Delta t\) is the time interval over which the displacements \(\Delta r\) were determined, \(D\) is the diffusion coefficient, and \(\sigma^2\) is the experimental uncertainty in bead position tracking.

LM interval speeds were calculated from overlapping displacements over each \(\Delta t = 10\) second interval between image frames by

\[
v = \frac{\Delta r}{\Delta t} = \frac{\sqrt{(x(t + \Delta t) - x(t))^2 + (y(t + \Delta t) - y(t))^2}}{\Delta t}.
\]  

(3)

The mean squared displacement was calculated in two ways denoted by \(\text{MSD}_{\text{TA}}\) and \(\text{MSD}_{\text{ETA}}\) [58]. The trajectory-averaged \(\text{MSD}_{\text{TA}}\) is computed independently for each trajectory and uses the mean of the squared displacements for each time lag \(\tau\):

\[
\text{MSD}_{\text{TA},j}(\tau) \equiv \left\langle \Delta r_j^2(\tau) \right\rangle = \frac{\Delta t}{T_j - \tau + \Delta t} \sum_{t=0}^{T_j-\tau} \Delta r_j^2(t, \tau).
\]  

(4)

The normalization prefactor is the inverse of the number of data points that contribute to the average for each \(\tau\). \(\Delta t = 10\) s is the time interval over which displacements are recorded in the experiment and \(T_j\) is the total duration of the \(j\)th trajectory; both \(T_j\) and \(\tau\) are integer multiples of \(\Delta t\). The \(\text{MSD}_{\text{ETA}}\) is computed over an ensemble of trajectories and is given by

\[
\text{MSD}_{\text{ETA}}(\tau) \equiv \left\langle \frac{\langle \Delta r^2(\tau) \rangle}{N} \right\rangle = \frac{1}{N} \sum_{j=1}^{N} \Delta r_j^2(\tau),
\]  

(5)

where \(N\) is the size of the ensemble.

Anomalous diffusion exponents \(\alpha\) were determined by the slope of a linear fit of log MSD versus log time lag, \(\tau\). MSD and hence \(\alpha\) were determined to a maximum time lag of \(\tau_{\text{max}} = 0.1T_{\text{msr}}\), where \(T_{\text{msr}}\) is the total measurement duration (12.5 hours for LMs on peptide lawns and 6.2 hours for LMs on bare lawns). We select \(\tau_{\text{max}}\) of 0.1 to avoid using larger lags where there are less time points to average over, as it has been shown that larger lags (approaching the trajectory length) have high variation for highly heterogeneous systems [59].

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The lawnmower: an artificial protein-based burnt-bridge molecular motor

Supporting Information

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1 Lawnmower design

The original LM concept and design was introduced by Kovacic et al. [?], though its motility was not tested. In their work, the LM consists of a quantum dot hub decorated with trypsin enzymes. Because of its small size, tracking quantum-dot motion requires fluorescence imaging. Its small size also means that it may penetrate polymer brush lawns, where it has been observed to undergo electrostatically mediated adhesion to the surface [?]. Furthermore, because Kovacic et al. determined that the multivalency of the QD LM is only \( N \sim 8 \) trypsins [?], its processivity and mechanochemical performance may be quite low [?, ?, ?]. Thus, for the work presented we implement the lawnmower design using a much larger micron-sized hub.

A schematic representation of the lawnmower and peptide lawn can be found in Fig. S1. The figure is not to scale. The Dynabead hub is 2.8 \( \mu m \) in diameter. Sulfo-SMCC tethers linking the trypsin to the hub are 8.3 Å in length. The density of amines on the Dynabead surface is \( \sim 40,000 \ \mu m^{-2} \) (manufacturer information), providing an estimate of the surface density of trypsin. The F127 (PEG\(_x\)-PPG\(_y\)-PEG\(_x\)) polymer brush was prepared with deposition conditions that produce an estimated density of PEG chains on the lawn of \( \sim 0.1 \text{PEG chains/nm}^2 \) [?].

Figure S1: (a) The lawnmower molecular motor concept based on a burnt-bridge ratchet design. In the current design the LM consists of a central Dynabead hub to which are bound trypsin proteases. (b) The triblock copolymer lawn chemistry is adapted from ref. [?]. Shown is a single NHS-F127 polymer, with NHS moieties on each of the terminal PEG groups. (c) A single ‘leg’ is comprised of a heterobifunctional polymer linker (sulfo-SMCC), here with \( N = 1 \) PEG units. (d) A ‘blade of grass’ from the lawn is the peptide substrate, with the trypsin cleavage site shown.
2 Motile and non-motile LM trajectories on peptide lawns

Figure S2: (a) The MSD$_{TA}$ of all LMs on peptide lawns throughout a single experiment. Trajectories are classified as motile or non-motile based on a threshold of MSD$_{TA} = 10 \, \mu m^2$ at $\tau = 4400$ s (the longest time lag plotted and used for all MSD analysis of LMs on peptide lawns). (b) A plot of all non-motile trajectories in one 12.5 hr experiment, centred at a common origin. Because of their extensive overlap, we concluded that the majority were irreversibly bound to the surface and thus used the average of these displacements as a stage drift trajectory. The drift trajectory was then subtracted from each motile LM’s trajectory to correct for sample drift, prior to further analysis. In panel b, each non-motile trajectory is plotted with a unique colour to help visually distinguish between trajectories.
3 $\Delta r$ analysis of LMs on peptide and bare lawns

Figure S3: $\Delta r$ analysis of LMs on peptide lawns. Similar to Figure 4a in the main text. Colours match those in Figure 2a of the main text. Image frames are recorded every 10 seconds.
Figure S4: $\Delta r$ analysis of LMs on bare lawns. Similar to Figure 4c in the main text. Colours match those in Figure 2b of the main text. Image frames are recorded every 10 seconds.
4 Diffusion analysis on bare lawns

4.1 Slightly subdiffusive $\alpha$ on a bare lawn

We hypothesized that the bare F127 track, not having peptide functionalized to its surface, would result in purely Brownian motion, and so were rather perplexed by the LMs displaying an average $\alpha$ that was slightly subdiffusive (Fig. 2f of the main text). A careful comparison to the DNA-blocked monowheel reveals similar behaviour: Yehl et al. present an MSD analysis on blocked beads which yields almost perfect Brownian motion ($\alpha = 0.99$, Fig. 3c of ref. [?]); however, closer inspection of the $\alpha$ distribution presented in their supplementary material (Fig. S11) shows that the blocked-monowheel $\alpha$ values peak in the subdiffusive regime at a value of $\alpha \approx 0.8$ and then tail off towards $\alpha \approx 1.0$. Thus, there appears to be a subdiffusive trend for their blocked beads as well. Although the components of the monowheel are distinct from the LM (the monowheel is a micron-sized DNA-coated bead moving on a lawn of RNA), it is interesting to speculate that a polymer-coated micron-sized bead diffusing on any lawn of substrate may exhibit slightly subdiffusive motion. It may be that heterogeneities in the lawn lead to local and partial obstructions to the particle motion. This hypothesis is supported by work on subdiffusive dynamics, where it has been shown that subdiffusion can result from partial obstruction of particle motion [?]. A careful analysis of local brush morphology would clarify this point, but is beyond the scope of the current work.

4.2 Diffusion coefficients

In order to extract the diffusion coefficient from Eq. 2 of the main text, the displacement distributions $P(\Delta r, \Delta t)$ are computed at increasing $\Delta t$ intervals, and the linear function $D \Delta t + \sigma^2$ found through fitting to Eq. 2 is used to determine the diffusion coefficient for that LM. One such fit for the LM trajectory shown in Fig. 4c of the main text is shown in Fig. S5a. The diffusion coefficient is determined in this way for all LMs on bare lawns ($n = 55$) and the resulting distribution of diffusion coefficients is shown in Fig. S5b. The average diffusion coefficient is $0.056 \pm 0.004 \mu m^2 \cdot s^{-1}$ and the average $\sigma^2$ is $0.0024 \pm 0.0017 \mu m^2$, where errors represent the standard deviation among measurements.
Figure S5: (a) Displacement distributions at different $\Delta t$ fit with the Rayleigh distribution for 2D diffusion (Eq. 2) provide estimates of $D\Delta t + \sigma^2$. For this trajectory, we find $D = 0.051 \, \mu m^2 \cdot s^{-1}$ and $\sigma^2 = 0.0051 \, \mu m^2$. (b) Distribution of diffusion coefficients for LMs on bare F127, where $D$ is determined for each trajectory as in panel (a).

A diffusion coefficient of $D = 0.056 \, \mu m^2 \cdot s^{-1}$ for these Dynabeads is less than that predicted by the Stokes-Einstein equation for diffusion of a sphere free in solution,

$$D_0 = \frac{k_B T}{6\pi \eta r},$$

where $T$ is temperature, $\eta$ the viscosity of water, and $r$ the particle radius ($1.4 \, \mu m$). Eq. 1 predicts $D_0 = 0.18 \, \mu m^2 \cdot s^{-1}$. Boundary walls (such as a planar surface in our experiments) influence the fluid flow around an object leading to an increase in drag, and therefore a decrease in diffusion coefficient. It is therefore expected that our diffusion coefficients are lower than predicted by Eq. 1. The correction to the diffusion coefficient for a sphere diffusing parallel to a boundary was developed by Faxén and is

$$D_{F,||} = D_0 \left(1 - \frac{9}{16} \frac{r}{h} + \frac{1}{8} \frac{r^3}{h^3} + \frac{45}{25} \frac{r^4}{h^4} + O\left(\frac{r^5}{h^5}\right)\right),$$

where $r$ is the particle radius, $h$ is the height from the surface, and $D_{F,||}$ the corrected diffusion coefficient.

Using Eq. 2 for our beads of radius $1.4 \, \mu m$ that are effectively touching the surface, we calculate that $D_{F,||}$ is expected to be $\sim 1/3$ of the bulk value predicted from Eq. 1. This agrees favourably with the ratio of our measured diffusion coefficient to the predicted bulk value, further supporting our conclusion that LMs are undergoing two-dimensional diffusion on bare lawns.
Figure S6: An example of a LM trajectory displaying saltatory motion on a peptide lawn. This trajectory is reproduced from Fig. 2a. Examples of saltations and pauses have been highlighted with a grey box and ellipses, respectively.