

Bacterial Motility Patterns Vary Smoothly with Spatial Confinement and Disorder

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In unconfined environments, bacterial motility patterns directly reflect the internal states of the cell. Bacteria operating a run-and-tumble behavioral program swim forward when in a “run” state, and they are stalled in place when in a reorienting “tumble” state. However, in natural environments, motility dynamics are a convolution of bacterial behavior and physical constraints. Recent investigations showed that *Escherichia coli* swimming through highly confined porous media exhibit extended periods of “trapping” punctuated by forward “hops,” suggesting a potential shift in motility strategy. We introduce a microfluidic device to systematically explore bacterial movement in a range of spatially structured environments, bridging the extremes of unconfined and highly confined conditions. We show that run-and-tumble and hop-and-trap are not distinct locomotive modes, but end points of a continuous spectrum of motility. We present the first unifying framework, “swim-and-stall”, to characterize this continuum of observed motility patterns. We demonstrate that a single control program underlies motility across all environments tested—that is, physical structure alone shapes changes in observed output. Our results establish a quantitative link between behavioral rules and environmental context, and show that *E. coli* can navigate dynamic, complex habitats without reprogramming their motility strategy. This robustness may explain the evolutionary persistence of run-and-tumble behavior in a diverse range of peritrichously flagellated bacteria and inform broader models of active transport in structured media.

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I. INTRODUCTION

A diverse group of flagellated bacteria, including *Escherichia coli* and *Salmonella*, use a motility strategy called “run-and-tumble.” Briefly, bacteria swim forward when their flagellar filaments come together to form a rotating bundle (“runs”), and they reorient themselves when this bundle comes apart (“tumbles”). In a free solution, bacteria can execute this behavior with no environmental constraints. This produces trajectories that resemble a random walk, with ballistic runs in a persistently directed path punctuated by short tumbles that randomly reset the direction of motion [1–6].

E. coli in tightly packed environments display motility patterns strikingly different from those seen in unconfined

environments [7–9]. In highly confined hydrogels, bacteria remain “trapped” for long periods until they are able to reorient themselves to “hop” through an opening between pores [9,10]. What environmental factors contribute to the differences in motility dynamics? How do motility patterns transition between those observed in these two extreme cases? Intrinsic fluctuations in chemotactic signaling, such as variations in CheY-P levels, have been shown to produce broad distributions of run durations even in homogeneous environments [11]. Here, we ask a complementary question: to what extent do changes in motility statistics observed in structured environments arise from active modulation of internal control versus passive physical constraints? We introduce a microfluidic device to rigorously characterize the impact of environmental structure on *E. coli* motility patterns by systematically varying two parameters: confinement, a proxy for obstacle density, and disorder, a proxy for obstacle randomness (Fig. 1).

We find that *E. coli* trajectories reveal no sharp boundaries between distinct motility modes, but they vary continuously across environments. As confinement and disorder increase, population-averaged behavior transitions smoothly

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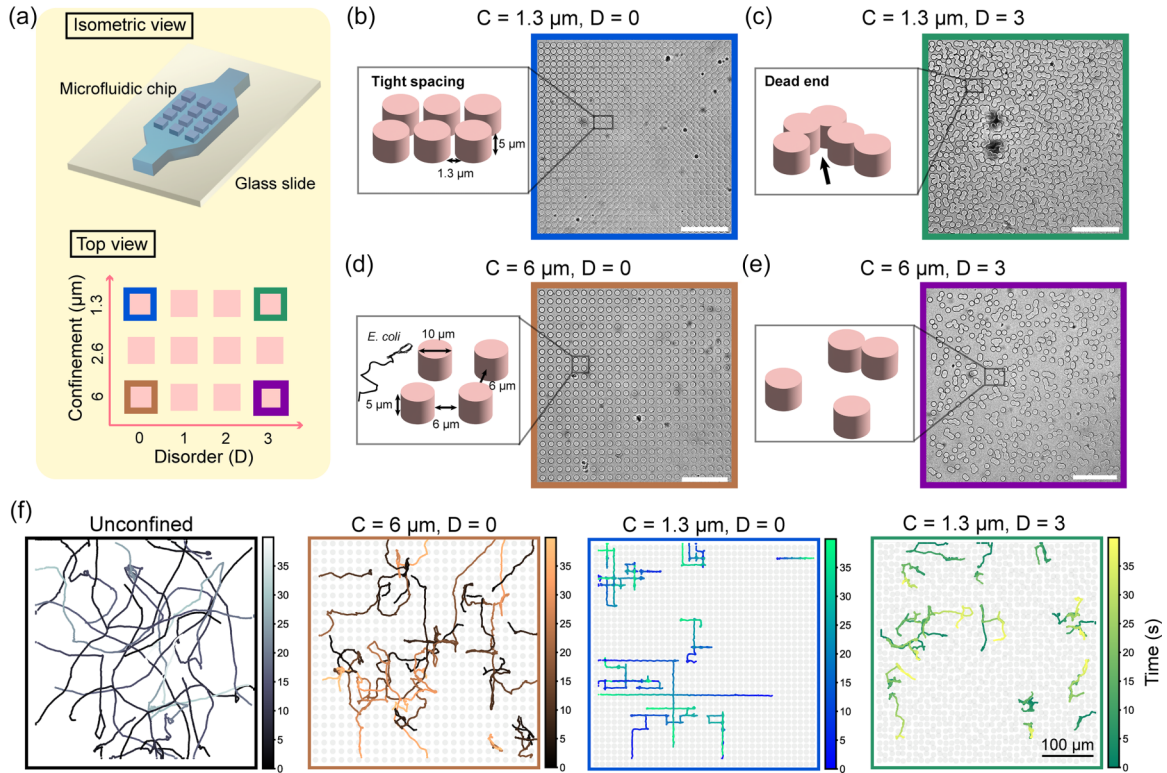


FIG. 1. A microfluidic device exposing bacteria to environments of varying levels of confinement and disorder. (a) Schematic of our experimental device in isometric and top views. The PDMS microfluidic chip comprises 12 regions, represented as pink rectangles. Each region exposes bacteria to a different level of confinement C and disorder D . The highlighted rectangles correspond to bright-field images of $C = 1.3 \mu\text{m}$, $D = 0$ shown in (b), $C = 1.3 \mu\text{m}$, $D = 3$ shown in (c), $C = 6 \mu\text{m}$, $D = 0$ shown in (d), and $C = 6 \mu\text{m}$, $D = 3$ shown in (e). Insets in (b)–(e) show examples of pillar geometry. The diameter of each pillar is uniformly $5 \mu\text{m}$ and the height is $10 \mu\text{m}$. Notable features of high confinement and high disorder are shown in (b) and (c). Tight spacing (b) and the dead end (c) exist when pillars are highly tightly packed and a few pillars merge to block the passage, respectively. (f) Representative tracked trajectories for unconfined and three structured regions [boxed in corresponding colors in (a)]. Twenty randomly selected trajectories are shown for each region; color gradient along the track indicates time lapse (darker to lighter coloring denotes time from start to end of the trajectory). Cells are tracked using the ImageJ plugin “TrackMate.” All regions are of size $400 \mu\text{m} \times 400 \mu\text{m}$; the unconfined region has no pillars.

from the run-and-tumble dynamics seen in unconfined swimming to the hop-and-trap dynamics observed in tightly packed environments. We formalize this continuum through a swim-and-stall framework that captures both regimes within a single description: “swims” correspond to directed motion (“runs” or “hops”), and “stalls” correspond to nonprocessive intervals between them (“tumbles” or “traps”). The framework is parametrized by two experimentally measurable quantities: an apparent tumble frequency, which determines swim duration, and a tumble-failure probability, which governs the likelihood of extended stalls. Together, these parameters capture how physical structure reshapes motility statistics without invoking distinct behavioral states.

Using this unifying formulation, we show that environmental geometry, not reprogramming of bacterial behavior, drives transitions in motility. We show evidence that bacteria in unconfined and highly confined environments are operating the same run-and-tumble motility program, just in different settings. Remarkably, this means that no change in intrinsic bacterial behavior (e.g., tumble frequency) is needed to transition across motility “modes” in different environments. We provide context for how quantifiable environmental constraints—confinement and disorder—

impact observed bacterial dynamics and show that environmental geometry is sufficient to explain changes in motility statistics across environments. This robustness likely reflects an adaptation to the wide range of environments that *E. coli* inhabits and moves through, ranging from open water such as lakes and rivers to dense heterogeneous environments such as intestinal mucus and soil [15,31]. Our results help explain the persistence of run-and-tumble as a general locomotion strategy across ecological contexts, and they establish a quantitative basis for modeling active transport in disordered systems.

II. RESULTS

A. A microfluidic system to investigate bacterial motility in spatially structured environments

We construct a microfluidic device to quantify how mechanical challenges posed by natural environments [12–15] affect *E. coli* motility patterns using two parameters: (i) confinement, a measure of the density of obstacles in a region; and (ii) disorder, the level of deviation from a perfect grid. Our quasi-2D device (height $5 \mu\text{m}$) comprises 12 confined regions and 1 unconfined region, each $400 \mu\text{m} \times 400 \mu\text{m}$. Confined

regions contain pillars of 5 μm height and 10 μm diameter in varying arrangements [Figs. 1(a)–1(e)]; the unconfined region contains no pillars.

Three levels of confinement C are considered: $C = 6\ \mu\text{m}$, 2.6 μm , and 1.3 μm , where C is the mean spacing between pillars. The chosen values of C range from the length of a flagellar filament ($\sim 5\text{--}10\ \mu\text{m}$) or the length of a larger cell in our sample ($\sim 6\ \mu\text{m}$) at the lowest level of confinement to the length of the smaller cells in our sample ($\sim 3\ \mu\text{m}$) and the width of the cell body ($\sim 0.5\text{--}1\ \mu\text{m}$) at higher levels of confinement [16–18]. For levels of disorder D , the center of each pillar is shifted away from the grid position by a value sampled from a uniform distribution with range $[-\frac{DC}{2}, \frac{DC}{2}]$; $D = 0$ corresponds to a perfect grid arrangement [Figs. 1(b)–1(e)]. For example, for the $C = 6\ \mu\text{m}$, $D = 0$ region, pillars are horizontally and vertically spaced 6 μm apart [Fig. 1(d)]; whereas for $C = 1.3\ \mu\text{m}$, $D = 3$, pillars are initially spaced 1.3 μm apart and their positions are displaced by $(dx, dy) \in [-\frac{3.9}{2}\ \mu\text{m}, \frac{3.9}{2}\ \mu\text{m}]$ [Fig. 1(c)]. Fluorescently labeled *E. coli* in a nutrient-rich medium are flowed into the device for observation; see Movies S1–S3 provided in the Supplemental Material [19].

Each trial records bacterial movement for 40 s at a time resolution of 0.05 s (20 frames per second). Randomly sampled trajectories from three structured conditions and in unconfined environments are shown in Fig. 1(f) ($n = 20$ trajectories from each region). We observe qualitative differences in bacterial

trajectories in structured environments and unconfined media [Fig. 1(f)]. We find that bacteria spend more time in confined regions than in the unconfined region ($t_{\text{total}} = 20.1\ \text{s}$ for $n = 110$ trajectories in unconfined versus $t_{\text{total}} = 22.5\ \text{s}$ for $n = 3991$ trajectories in confined environments, $p < 0.05$). Trajectories tend to be directed vertically or horizontally in confined, ordered regions, indicating that bacteria move along rows and columns of pillars rather than performing a uniform exploration of the space as in unconfined environments. Accordingly, we find that trajectories become more tortuous as disorder increases [Fig. 1(f) and Supplemental Fig. 1]. In the following sections, we quantitatively analyze these observations.

B. Swim-and-stall: A unifying framework across environments

Motility states for peritrichously flagellated bacteria (run versus tumble) have historically been thought to correspond directly to flagellar orientations (bundled versus unbundled) [2]. In free solution, there is no structural constraint to a bacterium expressing its internal state. However, the one-to-one mapping between internal and observed states can break down in complex environments [9,10]. Accordingly, a consistent framework for distinguishing between a bacterium's observed behavior and its internal state is crucial for making meaningful comparisons across conditions.

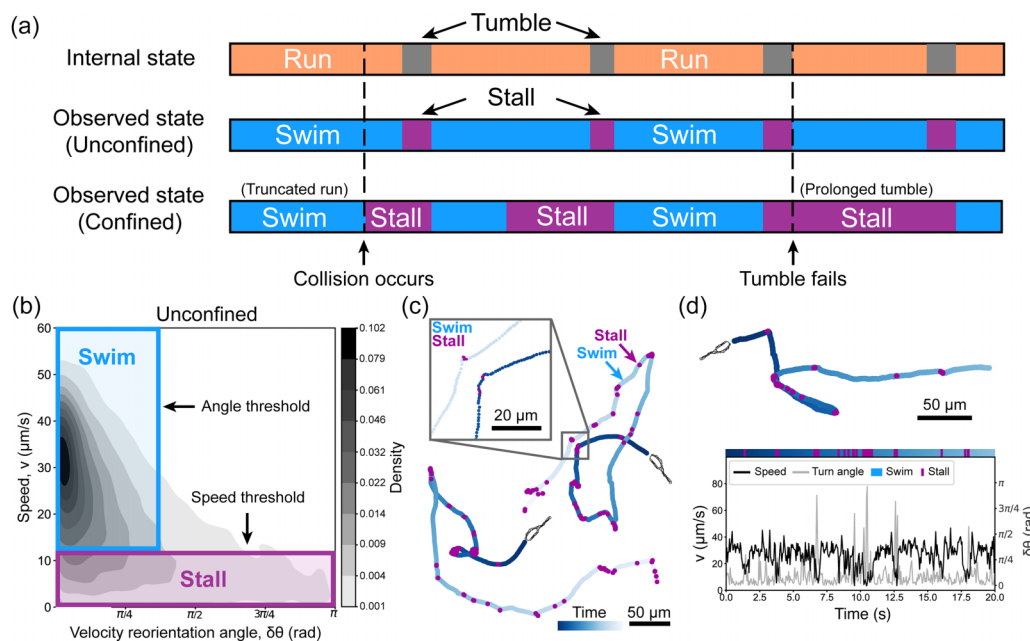


FIG. 2. Classification of bacterial trajectories. (a) Schematic demonstrating how bacteria employing the same run-and-tumble behavioral program (internal state, top) can display differences in observed state in unconfined regions (middle) and confined regions (bottom). First, collisions with obstacles in confined regions can result in swim periods that conclude before a full run is executed (see the first arrow at the bottom bar). Second, confined environments can trap a bacteria in place at the conclusion of a tumble, resulting in a prolonged stall period that comprises multiple internal state transitions (see the second arrow at the bottom bar). (b) The bivariate kernel density estimate shows the distributions of bacterial speeds v and turn angles $\delta\theta$ in the unconfined region. Swims (boxed in blue) occur when $v > 12.4\ \mu\text{m/s}$ (half the mean speed) and $\delta\theta < 3\pi$ rad. Stalls (boxed in purple) occur when the $v < 12.4\ \mu\text{m/s}$. (c) Two sample trajectories from bacteria in the unconfined region, with swims and stalls colored in blue and purple, respectively. (d) Twenty seconds of a sample trajectory in the unconfined region. Speed (black) and turn angle (gray) values for each frame determine the behavioral state (“swim” or “stall”) assigned to each frame. Color bar above shows a discrete sequence of states, with frames assigned as swims shown in blue and stalls in purple.

We introduce a unifying framework that classifies observed bacterial motion into two states: “swims,” which are periods of directed movement (corresponding to “runs” or “hops”), and “stalls,” which are nonprocessive periods (corresponding to “tumbles” or “traps”). These designations describe the observed dynamics of bacteria based on their center-of-mass (COM) trajectories, without direct knowledge of flagellar motor dynamics. In unconfined environments, observed and internal states align—all swims are runs, and all stalls are tumbles. However, in confined environments, obstacles can prematurely terminate a swim due to bacterial collisions with obstacles or extend a stall by physically trapping a cell [Fig. 2(a)].

Implementing a consistent discrete state mapping across conditions under this new framework allows us to quantify if and how motility patterns change in response to environmental structure. Swimming speeds in the unconfined region, $v(t) = |\dot{\mathbf{r}}(t)| \equiv |\dot{\mathbf{r}}(t + \delta t) - \dot{\mathbf{r}}(t)|/\delta t$, are narrowly distributed with mean speed $\langle v \rangle = 24.8 \mu\text{m/s}$, consistent with previously reported *E. coli* swimming speeds of 20–30 $\mu\text{m/s}$ [2,20]. Velocity reorientation angles (turn angles), $\delta\theta(t) \equiv \tan^{-1}\{|\dot{\mathbf{v}}(t) \times \dot{\mathbf{v}}(t + \delta t)|/[\dot{\mathbf{v}}(t) \cdot \dot{\mathbf{v}}(t + \delta t)]\}$, are broadly distributed from 0 to π [Fig. 2(b)]. We use the term “swim” period when a cell is moving at least half the mean unconfined speed, $|\dot{\mathbf{v}}(t)| \geq 12.4 \mu\text{m/s}$, and its turn angle is less than or equal to $\pi/3$ radians (60°). We define “a stall” period as one in which a cell moves slower than half of the mean unconfined speed; turn angles for stall periods are widely distributed [9]. This thresholding strategy was chosen for its consistency with prior work [9]. The structure observed in the joint speed-turn angle distribution [Fig. 2(b)] further supports that these two states are separable. These criteria ($|\dot{\mathbf{v}}| > 12.4 \mu\text{m/s}$, $\delta\theta > \pi/3$) ensure that fewer than 5% of frames remain unclassified, and results are robust to small threshold variations (Supplemental Fig. 2).

Representative trajectories in the unconfined region labeled according to these two states (swim and stall) are shown in Fig. 2(c). Cells alternate between periods of directed motion and in-place reorientation. A sample timecourse of swimming speed and turn angle further illustrates this pattern: swims are characterized by high swimming speeds and low turn angles, and stalls are characterized by precipitous drops in speed and spikes in turn angle [Fig. 2(d)].

C. Swim lengths decrease and stall bias increases with confinement

Using this swim-and-stall framework, we compare observed motility dynamics across different levels of confinement and disorder. For each condition, we compute the swim time $\langle T_{\text{swim}} \rangle$ and the mean stall time $\langle T_{\text{stall}} \rangle$ of all tracked cells [Figs. 3(a) and 3(b)]. The mean swim time is highest in the unconfined region ($\langle T_{\text{swim}} \rangle = 0.77 \text{ s}$) and lowest in the most confined region ($\langle T_{\text{swim}} \rangle = 0.25 \text{ s}$ for $C = 1.3 \mu\text{m}$, $D = 0$). The swim time distribution for the unconfined region is significantly different from all other conditions ($p < 0.0001$, Supplemental Fig. 3). The level of disorder does not significantly affect $\langle T_{\text{swim}} \rangle$.

Stall times increase with both confinement and disorder; additionally, the variance in stall times increases in disordered regions [white circles in Fig. 3(b)]. Stall times in all structured environments are significantly different from those in unconfined environments ($p < 0.05$, Supplemental Fig. 4). We hypothesize that prolonged stalls comprise multiple state transitions that cannot be resolved experimentally [Figs. 2(a) and 3(b)]. For instance, several reorientations may be required for bacteria to move past dead ends created by disordered pillar array geometries. We also see a wider distribution of the turning angle in these regions (Supplemental Fig. 5).

The mean stall (observed tumble) bias, $\langle P_{\text{stall}} \rangle$, is the fraction of time at which cells stall on average, and it is defined as $\langle P_{\text{stall}} \rangle \equiv \sum_{i=1}^N [n_{\text{stall}}^i / (n_{\text{stall}}^i + n_{\text{swim}}^i)] / N$, where n_{stall}^i and n_{swim}^i are the number of frames assigned to stall or swim states of the i th cell, respectively, and N is the total number of tracked bacteria. $\langle P_{\text{stall}} \rangle$ is much higher in structured regions as compared to unconfined media [Fig. 3(c)], supporting the hypothesis that cells show shorter swim durations when they encounter physical obstacles (i.e., they have a higher apparent tumble frequency). The increase in stall bias observed in structured environments likely arises from changes in both swim and stall duration.

Together, these results show that both swim and stall statistics vary smoothly with confinement and disorder, suggesting that run-and-tumble and hop-and-trap dynamics lie along a motility continuum with no sharp transitions between discrete motility modes. To further quantify directional persistence across conditions, we compute the velocity autocorrelation

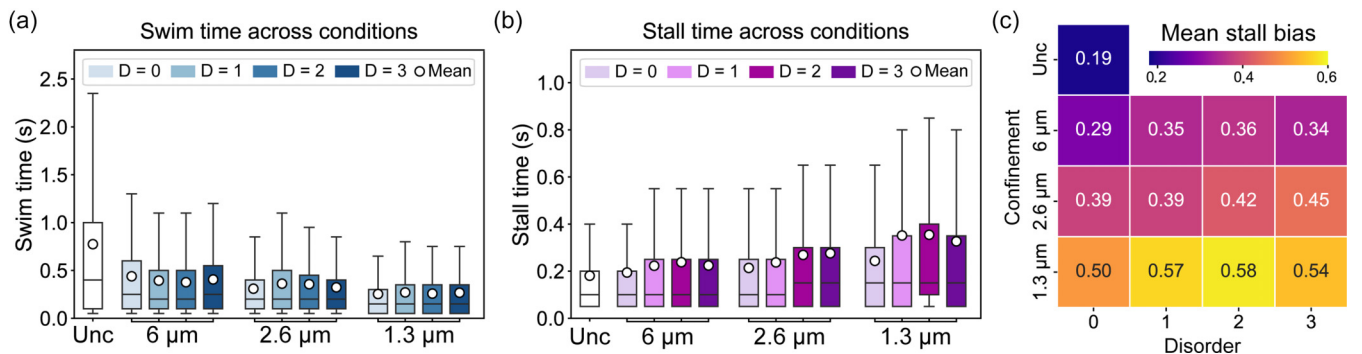


FIG. 3. Dynamics of swims and stalls. (a) Swim time and (b) stall time distributions across experimental conditions shown in boxplots. All distributions are log normal with positive skew. The lines inside the boxes indicate the median values, and white circles mark the mean values. (c) The heatmap of mean stall bias across experimental conditions of confinement C and disorder D . Mean stall bias is defined as the average stall time proportion over all tracked bacteria in a given region. The label “unc” represents the unconfined region.

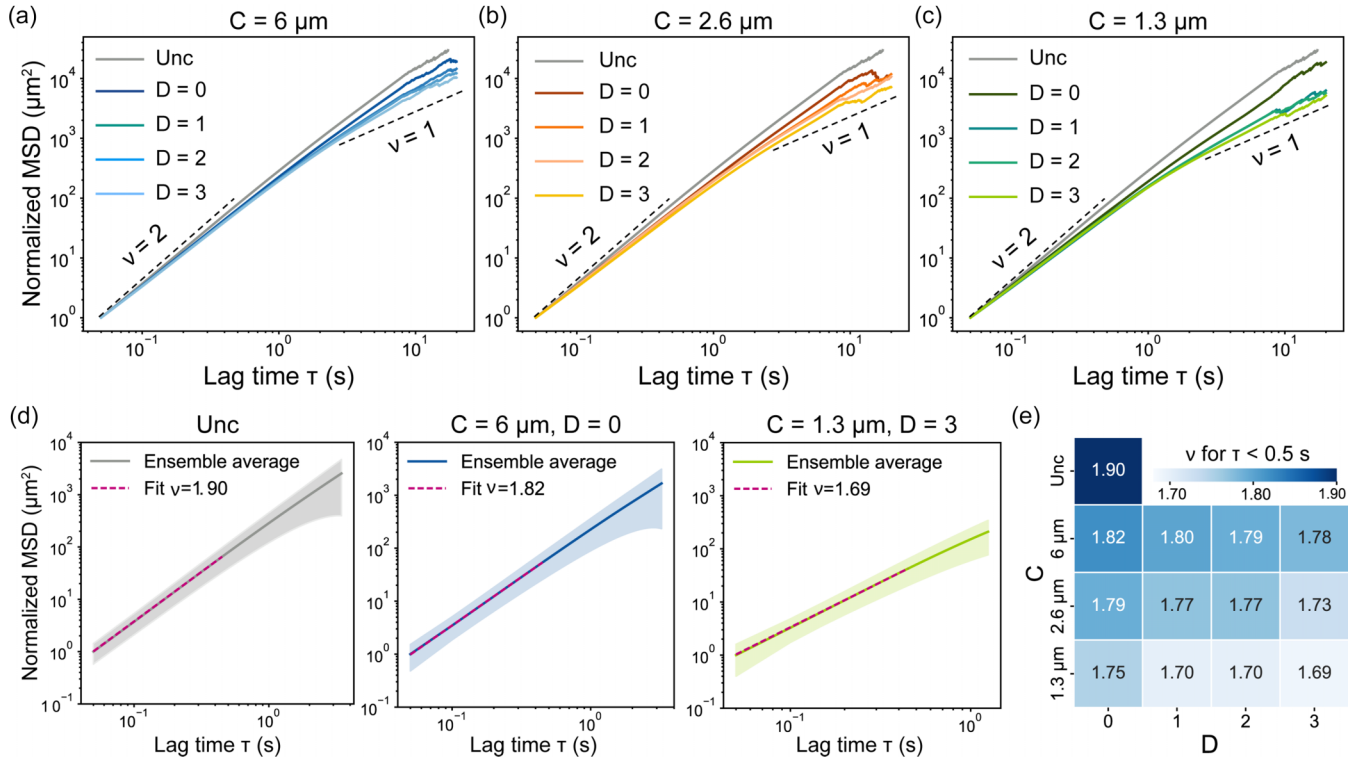


FIG. 4. Long-range dynamics across varying levels of confinement and disorder. (a) Normalized ensemble-averaged mean square displacements (eMSDs) for $C = 6 \mu\text{m}$. MSDs are normalized by dividing by the value at $\tau = 0$. Each plot depicts eMSD curves for varying levels of disorder D . Dashed lines show $\sim t^\nu$ for ballistic ($\nu = 2$) and diffusive motion ($\nu = 1$). Corresponding non-normalized MSDs are provided in in Figs. S4 and S6. (b) The plot of eMSDs for $C = 2.6 \mu\text{m}$ with varying the levels of disorder. (c) The plot of eMSDs for $C = 1.3 \mu\text{m}$. (d) Curves for eMSDs at early lag times for unconfined (left), $C = 6 \mu\text{m}$; $D = 0$ (center), and $C = 1.3 \mu\text{m}$, $D = 3$ (right). Dashed pink lines show fits estimating ν for $\tau < 0.5$ s. The error bands indicate $\pm$ one standard deviation. (e) Fitted values of ν for $\tau < 0.5$ s across confinement C and disorder D .

function (VACF) $C(\tau) = \langle v(t)v(t+\tau) \rangle / \langle v^2 \rangle$ for trajectories in each condition (Fig. S6). Correlations decay progressively faster and lose single-exponential character with increasing confinement and disorder, consistent with a smooth, continuous change in motility statistics rather than distinct motility modes.

D. Characteristic patterns coexist in complex structured environments

In free solution, bacteria express their internal states with no structural constraints: bacteria exhibiting a run-and-tumble behavior show ballistic swims punctuated by short reorienting stall periods [2]. These dynamics are captured clearly by examining the mean-squared displacements, $\text{MSD}(t) = \langle [r(t+\tau) - r(t)]^2 \rangle$. The ensemble-averaged MSD for run-and-tumble behavior in unconfined environments varies quadratically ($\nu = 2$) for short lag times τ , reflecting ballistic dynamics during runs (swims), and linearly ($\nu = 1$) at long lag times, reflecting long-term diffusive dynamics. The crossover time between these two regimes τ_c reflects the decay of directional persistence, which occurs over timescales longer than a single swim period due to partial reorientation during tumbles [Figs. 4(a)–4(c)]. Hop-and-trap behavior deviates from that of unconstrained run-and-tumble both at short and intermediate timescales [9]. First, at short time lags,

bacteria in highly confined media display superdiffusive ($1.5 < \nu < 2$), rather than ballistic, dynamics [Fig. 4(d)]. Second, at intermediate time lags, bacteria display transient subdiffusive ($\nu < 1$) dynamics; these give way to diffusive behavior at longer timescales as in unconstrained run-and-tumble behavior.

We observe bacterial trajectories consistent with both extremes in all structured environments considered. Close examination of individual MSDs reveals a high level of variance in structured regions, with the proportion of trajectories displaying ballistic dynamics decreasing as confinement and disorder increase (Supplemental Figs. 7 and 8). The value of ν for short time lags decreases as confinement and disorder increase, and ensemble dynamics approach those consistent with the hop-and-trap model [Fig. 4(e)] [9]. Accordingly, ensemble dynamics at short time lags for bacteria in the most confined and most disordered region skew closest to those expected from hop-and-trap behavior ($\nu = 1.69$ for $C = 1.3 \mu\text{m}$, $D = 3$).

We similarly observe trajectories that show apparent subdiffusive behavior at intermediate time lags, with the proportion of such trajectories increasing with both confinement and disorder (Supplemental Figs. 8 and 9). However, this trend can easily be masked when the variance in the dynamics is large, as is the case at intermediate levels of confinement. This is consistent with previous observations that ensemble-averaged

values of ν remain close to 1 until packing is very dense in porous media [9].

E. Both swim and stall duration tune transitions between motility patterns

Our experiments show that environmental structure has two major impacts on bacterial motility patterns. First, swim periods are shortened as cells collide with obstacles [Fig. 3(a)]. Second, stall periods are lengthened due to cells becoming “trapped” by tight, tortuous pore spaces [Fig. 3(b)]. To untangle the effects of these changes on observed ensemble dynamics, we define two parameters: (i) apparent tumble frequency F_{tumble} controls the duration of swim periods; (ii) tumble failure probability P_{fail} controls the duration of stall periods.

We perform simple run-and-tumble simulations to disentangle the effects of varying apparent tumble frequency and tumble failure probability on motility dynamics. Run-and-tumble durations are drawn from exponential distributions; tumble failure corresponds to a failed reorientation event that produces a nonprogressive run. Our simulations confirm that varying F_{tumble} (changing swim duration) tunes the length of time that ballistic dynamics are observed; shorter swims (e.g., due to collisions with obstacles) result in a lower crossover time between ballistic and diffusive dynamics. Our experiments show that bacteria implementing run-and-tumble behavior in unconfined media show low apparent tumble frequencies, with swim duration far exceeding stall duration [Fig. 3(b), Supplemental Fig. 10]. In highly confined environments, bacteria have been shown to exhibit far shorter swim (“hop”) durations [9,10]. At such high apparent tumble frequencies ($\langle T_{\text{swim}} \rangle = 0.12$ s when $F_{\text{tumble}} = 8.0$ in our model), swim periods may be so short that the ballistic region is difficult to resolve at experimental resolution ($dt = 0.05$ s). Our simulations show that this is sufficient to generate superdiffusive behavior at small time lags [Figs. 5(a) and 5(b)], as is noted in hop-and-trap dynamics (Supplemental Fig. 11, top left panel).

On the other hand, the appearance of subdiffusive dynamics at intermediate time lags requires lengthening the stall duration [9]. Our simulations do not show subdiffusive dynamics even at the highest apparent tumble frequencies when tumble failure probability $P_{\text{fail}} = 0$ (Supplemental Fig. 11, top left panel). Transient subdiffusion has been observed in systems with long trapping periods within heterogeneous environments [21–24]; in bacterial systems, we might expect such subdiffusive behavior because cells are “trapped” in place during prolonged stall periods comprising multiple transitions when tumble failure probability is high [Fig. 2(a)]. In our simulations, we observe an increase in the proportion of trajectories displaying transient subdiffusive behavior as P_{fail} increases and cells are more likely to show longer stall (prolonged tumble) periods [Figs. 5(c) and 5(e); Supplemental Fig. 12]. This is in agreement with our experimental results, which show high variance in the individual MSDs as environmental confinement and disorder are introduced [Figs. 5(d) and 5(f); Supplemental Figs. 7 and 8]. Additionally, transient subdiffusion at the ensemble level appears only at high values of P_{fail} [Fig. 5(b); Supplemental Fig. 12]. This supports

the absence of ensemble-level subdiffusive behavior in our experiments (Fig. 4), and aligns with findings that transient subdiffusion emerges only when stall durations significantly exceed swim durations [9].

F. Environmental structure affects observed, but not internal, states

While environmental structure clearly affects observed motility patterns, it remains unclear whether it also affects transitions between *internal states*. That is, do individual bacteria adapt their motility strategy (e.g., vary the frequency rate at which tumbles are attempted or concluded) to improve performance as they receive mechanical feedback from their environment? Or are the changes in apparent tumble frequency and tumble failure probability we characterize above mainly driven by environmental constraints (e.g., collisions and trapping) with the underlying run-and-tumble behavioral program remaining consistent across conditions [Fig. 2(a)]?

To address these questions, we calculate the distribution of conditioned swim lengths that approximates the swim lengths expected if bacteria do not change their underlying behavioral program—that is, if apparent tumble frequency only changes due to collisions with an obstacle. Here, we define *swim length* as the distance traversed during a swim period. This distribution is computed by considering the distribution of unconstrained swim lengths and the distribution of chord lengths—the length of free straight paths starting from randomly chosen points within a given pillar geometry—and the minimum of the two random lengths is taken [25,26] [Figs. 6(a)–6(c)]. The chord length distribution describes the straight paths available to bacteria given a randomly chosen initial position and a random direction (Supplemental Fig. 13).

We find that observed swim lengths match the conditioned swim lengths well in disordered environments with low to intermediate confinement (e.g., for $C = 6$ μm , $D = 3$ and $C = 2.6$ μm , $D = 3$, $R^2 = 0.998$ and 0.983 , respectively). This strongly indicates that there is no change to the underlying behavioral program of bacteria as they are navigating these environments. In highly confined environments, the observed distribution of swim lengths aligns most closely with the chord length distribution (e.g., for $C = 1.3$ μm , $D = 0$ and $C = 1.3$ μm , $D = 3$, $R^2 = 0.978$ and 0.963 , respectively), suggesting that the availability of free straight paths is mainly responsible for constraining swim lengths in this regime. This is again a strong indicator that the geometry of the environment, rather than bacterial behavior, is the primary feature driving changes in observed motility patterns. Similarly, in environments with intermediate confinement and high order ($C = 2.6$ μm , $D = 0$), we find that the observed distribution matches our prediction closely for short swim lengths but matches the chord length distribution for longer swim lengths [Figs. 6(a)–6(c)].

III. DISCUSSION

Environmental structure can interfere with the unconstrained execution of a bacterium’s behavioral program. We present the first unifying framework for characterizing *E.*

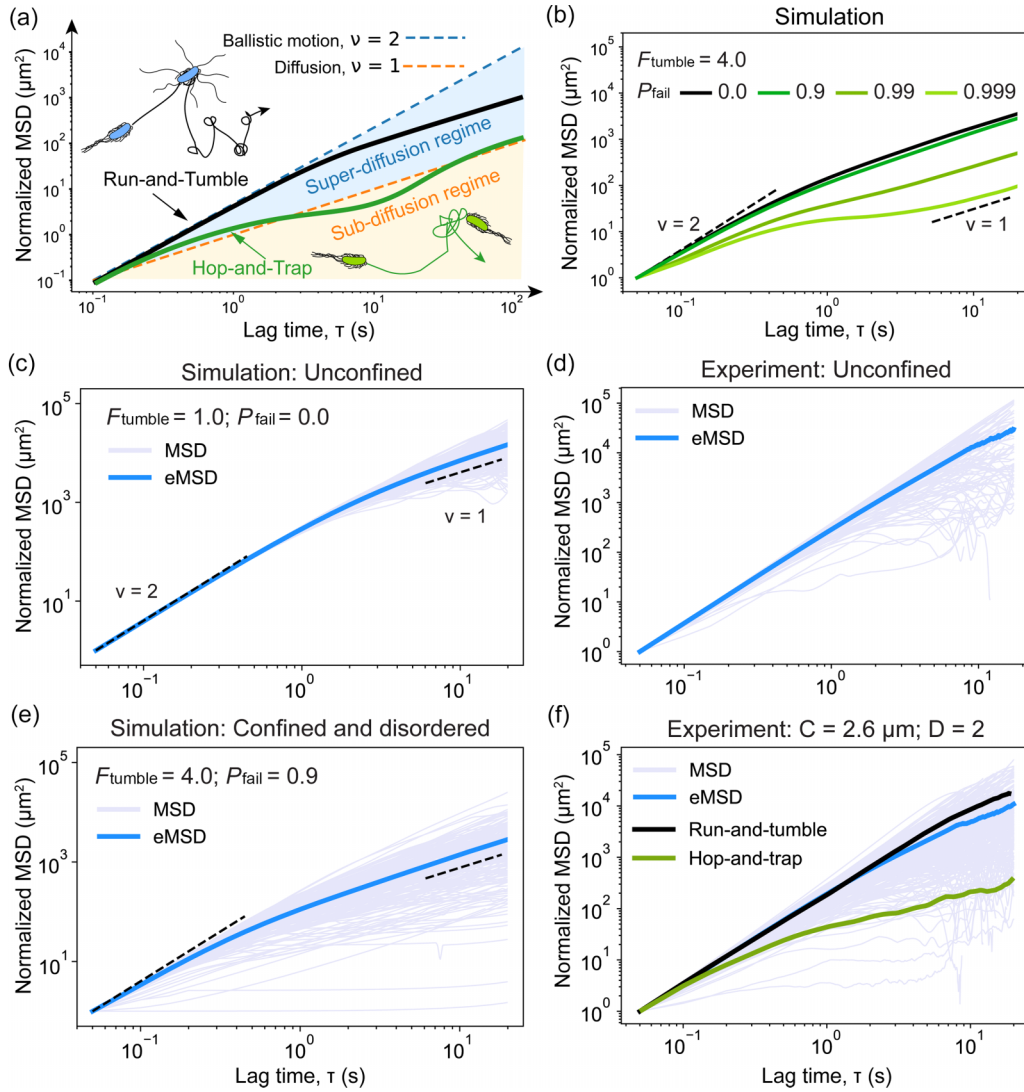


FIG. 5. Characteristic motility patterns coexist in structured environments. (a) Schematic of characteristic MSD curves for observed dynamics for bacteria employing unconstrained run-and-tumble behavior and observed dynamics under the hop-and-trap model. (b) Normalized ensemble-averaged MSD plot of ensemble dynamics with $F_{\text{tumble}} = 0$ and varying tumble failure, P_{fail} . MSDs are normalized by dividing by the value at $\tau = 0$. (c) Time-averaged MSDs (in lavender) and ensemble-averaged MSDs (in blue) from simulations. Simulation parameters are chosen according to those observed in the unconfined region, $P_{\text{fail}} = 0.0$ and $F_{\text{tumble}} = 1.0$. (d) Time-averaged MSDs (in lavender) and ensemble-averaged MSDs (in blue) from experiments in the unconfined region. (e) Time-averaged MSDs (in lavender) and ensemble-averaged MSDs (in blue) from simulations in a confined and disordered region, $P_{\text{fail}} = 0.9$ and $F_{\text{tumble}} = 4.0$ (bottom left). (f) Time-averaged MSDs (in lavender) and ensemble-averaged MSDs (in blue) from experiments in the region $C = 2.6 \mu\text{m}$, $D = 2$. Experimental trajectories highlighted in black and green are representative of expected run-and-tumble and hop-and-trap dynamics.

coli motility patterns across a diversity of environments, from a free solution to highly confined and disordered media [Fig. 7(a)]. Within this “swim-and-stall” framework, “swims” are periods of directed forward movement (whether terminated by an internal behavioral transition or an external collision), and “stalls” are nonprocessive periods between adjacent swims (whether they reflect tumbles or mechanical trapping). We show that observed motility patterns vary continuously across physical regimes (Figs. 7 and S6), and that *E. coli* motility adapts passively and robustly to environmental structure without requiring active retuning of its underlying control logic.

While both confinement C and disorder D shape environmental geometry, their influence on chord length distributions differs: increasing C compresses the accessible free path space more drastically than increasing D over the range explored. This likely accounts for the stronger dependence of $\langle T_{\text{swim}} \rangle$ on C than on D , despite observable changes in chord length distributions across all conditions. We also note that while our environments span a wide range of structural complexity, the smallest obstacle spacings remain below the mean run length of unconfined *E. coli* [2]. As such, we do not expect cells to exhibit fully unperturbed run-and-tumble behavior even in our least structured geometries. Extending the upper end of this continuum—e.g., using obstacle arrays with

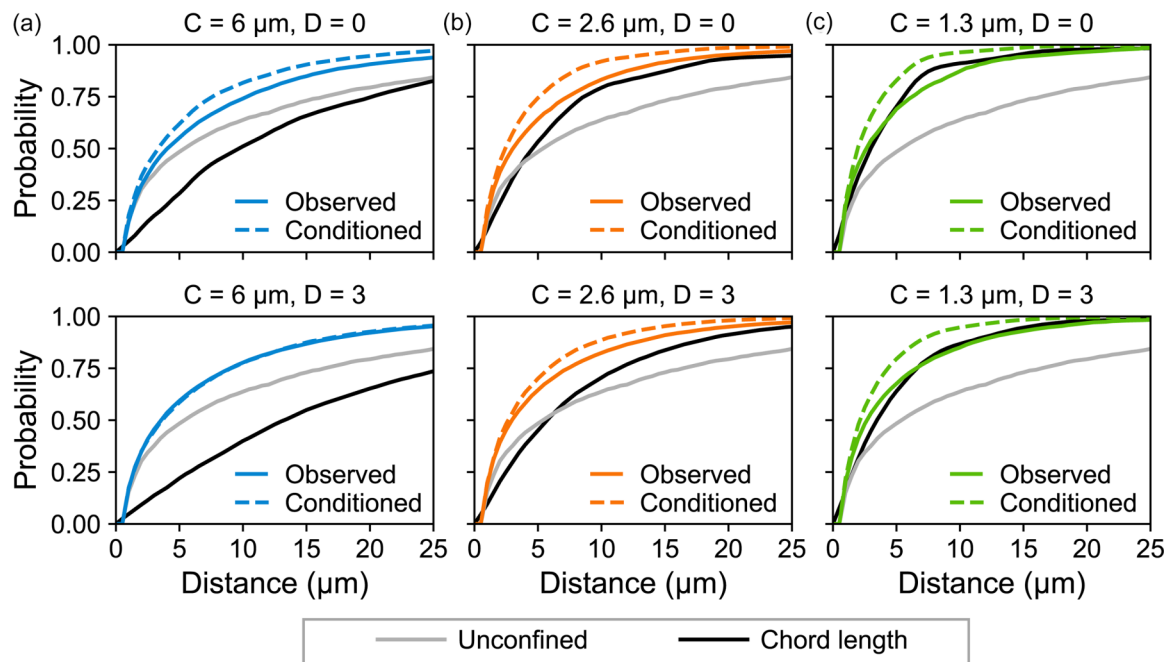


FIG. 6. Observed and conditioned swim lengths for bacteria employing swim-and-stall behavior in confined and disordered environments. Cumulative distribution function (CDF) plots comparing observed [labeled in blue in (a), orange in (b), or green in (c)] and conditioned swim lengths (corresponding dashed lines) for *E. coli* across levels of confinement [increasing from (a) to (c)] and disorder (increasing from top to bottom). The CDFs for unconfined swim lengths (gray) and chord lengths (black) are also shown in each panel. The conditioned distribution corresponds to the minimum of the unconfined swim length and the chord lengths; this assumes that changes in swim lengths arise solely from environmental constraints, with no change in internal state transitions across conditions.

spacings exceeding typical run lengths—would help assess when motility dynamics become functionally equivalent to those in bulk. While the specific numerical values of C reflect the morphology and growth stage of the *E. coli* strain used here, the underlying framework can be scaled to other bacterial species by accounting for differences in body and flagellar dimensions. Future work incorporating nondimensional geometric parameters would help facilitate direct cross-species comparisons and further generalize this approach.

Our quasi-2D microfluidic platform allows precise, independent control over geometric parameters (like obstacle density and spatial disorder), which is an advantage not currently achievable in 3D porous media. However, this dimensional reduction also limits direct comparison to movement through 3D environments. Metrics like chord length distributions and mean free path statistics can serve as a first-order bridge to quantify geometric constraints on swimmer trajectories across both 2D and 3D systems.

As observed motility dynamics often represent an entanglement of bacterial behavior and environmental constraint, we investigate what drives changes in these motility parameters across conditions. We compute the distribution of swim lengths observed in the unconfined region conditioned on the geometry of each structured environment considered to investigate whether *E. coli* modify their behavior in response to changes in environmental structure (Fig. 6). At low and intermediate levels of confinement, we find that our predicted distribution matches well with observed swim lengths. At the highest level of confinement and disorder, we find that trajectories tend to mirror environmental geometry, with the

distribution of observed swim lengths closely matching the distribution of possible straight paths. Deviations between the conditioned and observed distributions in highly confined and highly ordered environments likely stem from our simplifying assumptions: (i) that all positions are equally likely to initiate a swim, and (ii) that all directions are equally likely to be chosen. In highly confined regions, the presence of dead ends can lead to localized trapping, producing highly nonuniform trajectory densities. In highly ordered arrays, shallow collision angles may promote surface alignment, and narrow obstacle spacing can restrict reorientation during tumbles [27,28]. Future work—for instance, by varying obstacle shape—could disentangle how steering and boundary interactions shape motility at high obstacle density.

Our results suggest that, in extreme environments, bacterial “decisions” may be largely overruled by environmental constraints, and that observed motility patterns in such environments may be independent of changes in the underlying run-and-tumble behavioral program. For instance, a “stalled” bacterium may not be able to display forward movement (i.e., enter a swim period) after concluding a tumble ($P_{\text{fail}} > 0$). In this case, the internal state and the observed state of a cell during a stall period deviate—that is, a single resolvable stall period may actually comprise multiple internal state transitions due to “failed tumbles” [Fig. 2(a)]. In the case of a single failed reorientation, a stall would be composed of a tumble (resulting in a failed reorientation), a nonprogressive run, and a second tumble (resulting in a successful reorientation). Prolonged stall periods may include multiple failed transitions between actual tumbles and nonprogressive runs.

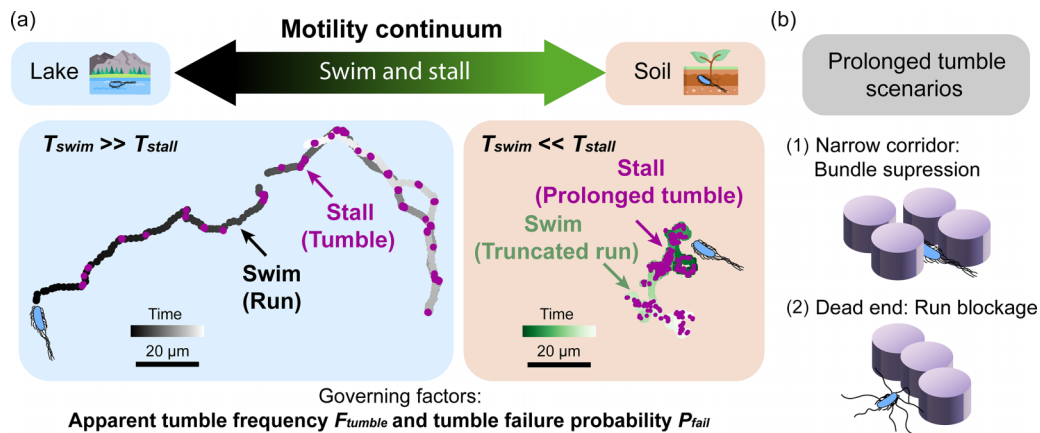


FIG. 7. Bacteria executing run-and-tumble behavior display a continuum of motility patterns tuned by duration of swim and stall periods. (a) Two highlighted trajectories are typical of run-and-tumble and hop-and-trap models corresponding to MSD curves in Fig. 5(c) with $C = 2.6 \mu\text{m}$; $D = 2$. (b) Two scenarios where stall periods might be prolonged due to environmental factors: (1) the flagellar bundle is suppressed in confined environments; (2) dead end blocks forward movement after reorientation in disordered environments.

Tumble failures occur in two environmentally driven situations [Fig. 7(b)]: (i) tight spacing forces the flagellar bundle to stay together, suppressing reorientation (high confinement), or (ii) a reorientation does not allow the subsequent run to be progressive because of an immediate collision with another obstacle within a “dead end” cluster (high disorder) [9].

While our results are consistent with a conserved run-and-tumble program operating across environments, direct measurements of motor activity or flagellar conformations in structured settings would be necessary to conclusively demonstrate behavioral invariance. Distinguishing conclusively between behavioral transitions and mechanical interruptions remains an open question due to challenges associated with imaging flagella in high yield assays [1,29]; direct visualization of flagellar orientation in swimming bacteria—particularly in complex environments—is a critical next step. The development of a more comprehensive simulation framework that explicitly models agent behavior and physical interactions is also a potential direction for future work. Models that incorporate full agent-based behavior, such as reorientation dynamics, rotational diffusion, or detailed motor-state transitions, may be useful to directly test the sufficiency of internal-state invariance in generating the full spectrum of observed motility patterns.

Previous studies have shown that intrinsic motor-level variability (e.g., through CheY-P fluctuations) can modulate run duration distributions in unconfined environments [11]. Our results highlight that altered motility statistics of *E. coli* swimming in structured environments reflect primarily physical constraints rather than active modulation of internal motor states. We note that these results focus on intermediate timescales (seconds to minutes); coupling between environmental interactions and internal behavioral states may emerge over longer timescales [30]. Further exploring how *E. coli* respond to sustained environmental cues is an important direction for future work.

E. coli typically inhabit dynamic and heterogeneous environments (e.g., biological tissue or complex fluids) [5,15,31], in which mechanical interactions with boundaries and obstacles may change on timescales far shorter than those

associated with long-term adaptation. Our framework captures this regime that is relevant to their natural behavior: rapid responses to fast fluctuations in environmental geometry. Excitingly, as supported by recent theoretical and experimental studies [32–34], our swim-stall framework may extend to other bacteria that employ run-and-tumble strategies. Confirming such generality will require testing across diverse species and environmental contexts.

Other strategies such as “run-and-reverse”—used primarily in marine bacteria like *Vibrio alginolyticus* and *Shewanella putrefaciens*—outshine run-and-tumble in specific contexts, such as adeptly responding to nutrient patches in the ocean by rapidly climbing chemical gradients [35,36]. This behavior (and its variants, including run-reverse-flick and run-reverse-wrap) can also be adapted to avoid getting trapped in tight spaces [37,38]. This is especially crucial for symbiotic species like *Vibrio fischeri*, as they must navigate to and through specific host tissues, often through highly confined and highly viscous regions [39–41]. While run-reverse strategies seem to prioritize success in structurally constant environments, the run-and-tumble motility program must be more broadly successful [25,38,42,43]. Indeed, when faced with complex or heterogeneous environments, *V. alginolyticus* grow lateral flagella and adopt a “generalist” strategy more akin to run-and-tumble [44]. Our work underscores how environment can shape our observation of a bacterium’s behavior, as well as how the range of environments a bacterium must successfully navigate can shape the evolution of its underlying behavioral program.

IV. MATERIALS AND METHODS

A. Strains and growth conditions

We grow a culture of wild-type *E. coli* (HCB33) with plasmids expressing red (mScarlet-I) fluorescent proteins on solid LB agar plates (LB Broth EZMix, Sigma-Aldrich + 1.5% Bacto Agar, MOLAR Chemicals). A single colony is subsequently inoculated in a 3 mL LB medium (LB Broth EZMix, Sigma-Aldrich) with 50 $\mu\text{g}/\text{mL}$ ampicillin and streptomycin for $16 \text{ h} \pm 30 \text{ min}$ at 30°C and 250 rpm. Overnight cultures

are backdiluted 1:10 000 in the same medium and grown to late exponential phase until it has reached an OD₆₀₀ of 0.5. Cell lengths were normally distributed with mean $\pm$ sd being $5.96 \pm 1.83 \mu\text{m}$, consistent with previous measures of RP437 in fast-growing conditions [18]. 10 μL of this culture is gently mixed with 990 μL of a fresh LB medium and antibiotics. The broad host-range plasmid, pMRE-145, was used in this study for constitutive production of mScarlet-I in conjunction with antibiotic resistance. For details on the plasmid, e.g., backbone, resistance markers, origin, and promoter, please refer to [45].

B. Microfluidic device fabrication and preparation

We design the pillar device using Klayout. We perform a direct write of the pillar device design on a 12.7-cm Cr mask plate (Heidelberg Mask Writer, DWL2000). We subsequently develop the patterns (Hamatech Mask Chrome Etch 1) and then place the mask plate for 2×10 min in a hot strip bath.

We prime a new Si wafer by spin-coating (3000 rpm/1000 rps/30 s) a thin layer of MicroPrime MP-P20 (ShinEtsu MicroSi, Inc.) to promote adhesion for photoresist. Next, we spin-coat Shipley 1813 photoresist on the wafer (using the same protocol) and immediately place it onto a 115 °C hot-plate for 60 s. We perform surface analysis (Filmetrics) to check for surface irregularities. The outer part (~ 1 cm) of the photoresist is removed from the edge of the wafer with an Edge Bead Removal System. Using ABM's High Resolution Mask Aligner, we expose the wafer for 3 s with near-UV (405 nm) through our developed mask. The wafer was subsequently developed using an MIF 726 (Hamatech-Steag wafer processor). After inspecting the wafer under a microscope, we subject it to a cycle of plasma descum to remove residual layers of photoresist (Anatech Resist Strip). We perform a Bosch fluorine process (Unaxis 770 Deep Silicon Etcher) to achieve a final etch depth of 5 μm , confirmed using the P-7 profilometer. We then strip the wafer of all resist using 2×10 min wet bath soaks. Finally, we use the MVD-100 molecular vapor deposition system with the antistiction coating, 1H,1H,2H,2H-perfluoro-octyltrichlorosilane (FOTS).

At this point, the wafer is ready to be used as a master mold of the pillar device. To make the microfluidic chips used for experiments, we mix polydimethylsiloxane (PDMS, Sylgard 184 Silicone Elastomer Kit, Dow Corning) in a ratio of 1:10, degas it in a desiccator, and pour it onto the master mold. We then bake it at 60 °C for 2 h. Devices were cut out from the wafer and we hole-punched 1.5-mm-diam holes at both inlets before binding them to a glass cover slip using an oxygen plasma oven.

C. Microfluidic experiments and imaging

We wet the device using an LB medium with antibiotics immediately after the post-bake upon plasma binding the device to a clean glass-bottom Petri dish. We then inoculate 1 μL of the OD₆₀₀ = 0.005 suspension (~ 1000 cells) in one inlet using a pipette tip. After inoculation, we seal both inlets using high-vacuum grease (Dow Corning). The sealed device is then submerged in Milli-Q water (preheated to 30 °C) to prevent

the device from drying out and placed onto the microscope stage for imaging. Due to the shallowness of the device, cells cannot be actively distributed in the pillar regions within the main channel at the time of inoculation. For this reason, a waiting time of ~ 2 h was determined from preliminary observations to lead to ideal cell density (10–50 cells in the field of view). During the time-lapse microscopy experiment, the temperature of the environment is maintained at 30 °C.

D. Recording and quantifying bacterial motion

Each device contains three levels of confinement for pillar regions and four levels of disorder for each level of confinement, amounting to a total of 12 unique pillar regions. Using a Nikon Eclipse Ti-E microscope equipped with a 20 $\times$ Plan Fluor objective, mCherry fluorescence (49 008, Chroma Inc.), and an Andor Neo sCMOS camera (Andor Technology plc.), we took video recordings for 40 s (20 fps, 800 frames) at 12 regions with varying arrangements of pillars and one unconfined region without pillars. We repeated the process a total of seven times over the course of ~ 30 min to ensure sufficient cell trajectories.

While the physiological state of the cells is going to be determined by time spent in the device, this is equal across all pillar regions, and given the short window of time needed for data acquisition, it is likely to remain relatively constant. Furthermore, given the time needed to proliferate, we observed a decrease in swimming activity when exposed to nongrowth motility buffers over this 2 h duration. To address this, a nutrient-rich medium was used.

We track between 68 and 605 cells for each region. To trace individual cells, we utilize the ImageJ plugin “Track-Mate” with subpixel precision [46]. All tracks are inspected for quality control. Repetitive tracks, tracks that do not show any sign of motion, and tracks that are shorter than 8 s (160 frames) before the cell exits the field-of-view are excluded. In addition, tracks of filamentous cells are filtered out to ensure that only cells in a healthy state are tracked. Raw tracking data (x, y, t) are exported from ImageJ, and further processing and analysis is performed using Python scripts. All scripts and protocols used for analysis are available on Github [47]. All raw images used for this research are publicly accessible in Dropbox [48].

Starting from the raw tracking data, we assign each frame of a trajectory to a “run” or “tumble” state based on a combination of speed and turning angle thresholds. Speed is calculated as $v(t) = |\vec{v}(t)| \equiv |\vec{r}(t + \delta t) - \vec{r}(t)|/\delta t$ and turning angle is defined as $\delta\theta(t) \equiv \tan^{-1}[\vec{v}(t) \times \vec{v}(t + \delta t)/\vec{v}(t) \cdot \vec{v}(t + \delta t)]$. We assign a frame as a run state when $v(t) \geq 12.4 \mu\text{m/s}$ (half the unconfined mean speed) and $\delta\theta(t) \leq \pi/3$ (i.e., 60°). To identify tumble states, we choose the speed cutoff at half mean speed so cells tumble at times when $v(t) \leq 12.4 \mu\text{m/s}$.

E. Chord length calculation

We use an in-house Python script to simulate chord lengths in all pillar regions (Supplemental Fig. 13). Chord lengths are defined as randomly sampled, simulated straight paths from randomly positioned origins to the first intersecting pillar.

To simulate chord lengths in each region, we recreate pillar arrangements for different levels of confinement and disorder. We then choose 400 randomly located origins from which 36 chords start; each chord ends at the first intersection with a pillar or edge of the device. A histogram of the resulting chords is used as an estimate of the probability density function of chord lengths in each region. Chord length distributions are not sensitive to the number of origins and the number of chords simulated.

F. Conditioned swim length distribution

Let p be the probability density function (pdf) of swim lengths without confinement, and let q be the pdf of chord lengths in a given environment. What is the expected swim length distribution if bacteria stopped when encountering a wall? If we independently draw one run length r with probability density $p(r)$ and one chord length s with probability density $q(s)$, the expected swim length in the environment is $z = \min(r, s)$. The pdf of the expected swim lengths is given by $f(z) = [1 - Q(z)]p(z) + [1 - P(z)]q(z)$, where Q and P are the cumulative distribution functions of chord lengths and free-space swim lengths, respectively. The conditioned swim length distributions shown in Fig. 6 are calculated from the empirically observed swim length distribution and the numerically determined chord length distributions using the formula above.

G. Mean-square displacement calculation

To compute mean-square displacements (MSDs), we divide tracks into nonoverlapping time segments ($dt = 0.05$ s, corresponding to one frame) as $\text{MSD}(\tau) = \langle [r(t + \tau) - r(t)]^2 \rangle$, where τ is the lag time. We compute both individual and ensemble MSDs. Individual MSDs are calculated by averaging squared displacements over time within each trajectory. To obtain ensemble MSDs, we use a trajectory-based approach: for each lag time, we average the MSD values of all individual trajectories with sufficient duration to contribute. All trajectories used in the ensemble MSD have at least 160 frames (8 s) to ensure reliable statistics. To ensure a reliable interpretation of the MSD results, we take several precautions to address known sources of bias and error that can affect the MSD curves. The range of τ is chosen to ensure that the minimum number of trajectories is at least 50, with a maximum τ of 20 s. We primarily interpret scaling behavior at early lag times where statistics are robust. MSD plots for both individual MSDs and ensemble MSDs (eMSDs) under all experimental conditions are shown in Supplemental Fig. 7. Normalized MSD plots are displayed in Supplemental Fig. 8. Normalization is done by dividing the MSD value at each time lag by the initial MSD value at time zero. This normalization facilitates a visual comparison across conditions by removing differences in absolute amplitude [49]. For completeness, all non-normalized MSDs are shown in the Supplemental Material. Furthermore, we fit eMSDs at early lag times ($\tau < 0.5$ s), as indicated in Fig. 4 and Supplemental Fig. 9. To fit eMSDs, we use a power law, $y = \beta x^\alpha$, where β and the exponent α are two parameters to adjust so as to produce the best fit, using the Python package SCIPY [50]. We present a heatmap to show how the exponent α varies across conditions [Fig. 4(e)].

H. Swim and stall simulations

We simulate stochastic run and tumble dynamics in two dimensions via an in-house Python script. Tumbles are initiated at a rate F_{tumble} per second; each tumble duration is drawn from an exponential distribution with mean $\langle T_{\text{tumble}} \rangle = 0.2$ s [Fig. 2(d)]. During each tumble, the cell instantaneously reorients to a new direction drawn uniformly from $[0, 2\pi)$. Tumble initiations are independent at each time point for each simulated bacterium. This results in an exponentially distributed run duration with mean $\langle T_{\text{run}} \rangle = 1/F_{\text{tumble}}$. At time points corresponding to the end of a tumble, another tumble is immediately initiated (corresponding to a failed reorientation) with probability P_{fail} . Simulations are performed at our experimental resolution, with $dt = 0.05$ s for $n = 200$ bacteria over a 60 s interval. MSDs for simulated trajectories are calculated as for experimental trajectories.

To test the robustness of our conclusions against deviations from exponential run statistics, we also implemented simulations incorporating time-correlated tumble initiation rates, which produce heavy-tailed run distributions consistent with experimentally observed rate variability (Fig. S6).

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There are no competing interests to declare.

DATA AVAILABILITY

The data that support the findings of this article are openly available [47].

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